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1. While the length of articles cannot be specified, they should not run to excessive length especially by inclusion of unessential tables and protocols which add materially to the cost of publication.

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THE AMERICAN JOURNAL OF PHYSIOLOGY

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NOTE ON THE EFFECT OF TEMPERATURE UPON THE ACTION OF THROMBIN AND ANTITHROMBIN

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From the Physiological Laboratory, Johns Hopkins University

Received for publication October 5, 1914

The main object of this note is to call attention to the marked augmenting effect of temperatures at or above the body-temperature upon the activity of antithrombin, and also to emphasize one important condition influencing the effect of high temperatures upon thrombin.

The effect of variations in temperature upon the time of coagulation has been studied by a number of observers, both as regards the clotting of normal blood and the coagulation of artificial plasmas or fibrinogen-solutions. In the case of normal blood the conditions are quite complex since several distinct processes come into play and these processes may be affected differently by the changes of temperature, the actual time of clotting being the resultant of their interaction. We have to consider, for instance, such changes as the disintegration of the platelets, the activation of the prothrombin, the neutralisation of the antithrombin and the final reaction between the thrombin and the fibrinogen. In consequence, perhaps, of this complexity of processes, and also because of the different methods employed, the results reported by different observers for normal blood have not been entirely concordant. All observers agree that between

0°C. and 20°C. rise of temperature is accompanied by a great acceleration of the time of clotting, the temperature coefficient for a range of 10°C. being quite large, 3 to 4. Between 20° and 30°C. some observers[1] find but little difference in the time of coagulation, while others,[2] making use of more delicate methods, state that there is a marked acceleration for a rise of temperature between these points, the temperature coefficient being approximately 2.5.

Between 30° and 40°C. there is a further difference in the reported results. Brodie and Russell got no variation in time of coagulation between these temperatures, Burker and also Addis found an acceleration, the temperature coefficient being from 1.4 to 1.7. Addis whose experiments were the most extensive states that beyond 42.5°C. coagulation is delayed, the temperature coefficient becoming negative. Observations upon artificial plasmas and upon fibrinogen-solutions have not given more uniform results, although the conditions involved would seem to be less complex than in shed blood. Rettger[3] states that with fibrinogen-solutions and thrombin (prepared by the method of Schmidt) the time of coagulation remains constant for variations of temperature between 17°C. and 41°C. Landsberg,[4] on the contrary, making use of similar preparations, reports that the time of coagulation is accelerated by rise of temperature up to an optimum which lies between 37° and 40°C. Beyond 40°C. the coagulation-time is somewhat delayed. My own observations tend to confirm the results reported by Landsberg. I made use of fibrinogen-solutions prepared in the usual way from oxalated plasma and thrombin solutions obtained by a method previously reported.[5]

It may be noted in this connection that the coagulation of fibrinogen-solutions by thrombin shows sometimes irregularities

[1] Lee and White: American Journal of the Medical Sciences, 1913, cxlv, 495.

[2] Brodie and Russell: Journal of Physiology, 1871, xxi, 403. Burker: Pflüger's Archiv, 1904, cii, 65. Addis: Quarterly Journal of Experimental Physiology, 1908, i, 305.

[3] Rettger: American Journal of Physiology, 1909, xxiv, 406.

[4] Landsberg: Biochemische Zeitschrift, 1913, l, 245.

[5] Howell: American Journal of Physiology, 1913, xxxii, 264.

which it is difficult or impossible to control, especially if minimal strengths of thrombin are used. If, for example, several mixtures of the same concentrations are made and are kept in a bath at a given temperature, it may happen that one of the specimens will vary markedly in its coagulation from the time shown by the majority of the preparations. Variations of this kind are due probably to some slight undetected difference in conditions whose influence is likely to be more evident the smaller the proportion of thrombin that is used.

In general, however, it was found that the optimum temperature of coagulation lies at 35°C. or between 30° and 35°, while at 40°C. there is a tendency toward a retardation or negative coefficient, which is never very marked and may be lacking in some cases, an important condition in this respect being again the proportional amounts of thrombin and fibrinogen used in the reaction. More decisive and interesting results were obtained in experiments upon the action of thrombin upon solutions of dried calcium-free blood plasma. The mode of making and using this dried plasma has been previously described[6] and need not be repeated here. With these solutions the time of coagulation is accelerated greatly between 0°C. and 20°C., the temperature coefficient being 3 or 3+, but between 20° and 35°C. the time of coagulation is not changed perceptibly. At 40°C. there is a very marked retardation—in fact, a permanent suspension of coagulation when the amount of thrombin used is not too large. This effect may be illustrated by the following experiment in which 1 drop of the thrombin solution was used to coagulate 10 drops of the plasma.

Temperature 20°C.	Coagulation-time between 5 and 10 minutes.
Temperature 25°C.	Coagulation-time between 5 and 10 minutes.
Temperature 30°C.	Coagulation-time between 5 and 10 minutes.
Temperature 35°C.	Coagulation-time between 5 and 10 minutes.
Temperature 40°C.	No coagulation within 3 hours, during which time the temperature of the bath was retained at 40°C.

[6] Howell: American Journal of Physiology, 1911, xxix, 187 and 1912, xxxi, i; also Archives of Internal Medicine, 1914, xiii, 76.

A specimen of the last solution removed after 2 hours to a temperature of 20°C. began to clot in 30 minutes and later formed a solid clot. Landsberg has observed a similar effect at body temperature in experiments made with a thrombin solution (Schmidt's method) and a magnesium sulphate plasma. His explanation is that the thrombin is adsorbed by some protein or proteins found in the plasma. In former papers I have given evidence for the existence of an antithrombin in blood-plasma, and the thought occurred that the striking difference in reaction at 40°C. between a thrombin-fibrinogen mixture on the one hand and a thrombin-plasma mixture on the other is due probably to the antithrombin present in the plasma. Direct experiments made to test this suggestion demonstrated that it is correct. In these experiments the action of the antithrombin was determined by its effect on selected mixtures of thrombin and fibrinogen exhibiting known coagulation times. For example, oxalated human blood plasma, freshly prepared, was heated to 60°C. and filtered. The antithrombin action of the filtrate was tested upon mixtures of thrombin and fibrinogen (dried plasma) at 20° and at 40°C. according to the following schema.

20°C. Thrombin solution drops	*Heated human plasma drop*	*Time interval minutes*	*Fibrinogen drops*	*Coagulation time minutes*
4	1	15	10	4 (solid)
3	1	15	10	5 (solid)
2	1	15	10	10 (partial)

Without the addition of the drop of heated plasma similar mixtures all clotted firmly in 4 minutes.

40°C. Thrombin solution drops	*Heated humam plasma drop*	*Time interval minutes*	*Fibrinogen drops*	*Coagulation time*
4	1	15	10	10 minutes (solid)
3	1	15	10	10 minutes (partial)
2	1	15	10	No clot in 5 hours.

This result was confirmed by other similar tests. It may be concluded that in mixtures in which the thrombin is not greatly in excess of the antithrombin an increase in temperature from 20°C. to 40°C. augments the action of the antithrombin to such an

extent that coagulation is greatly delayed or entirely prevented. In the plasma of circulating blood or lymph the prothrombin and antithrombin are present in such proportions that at room temperatures the coagulation of the cell-free plasma occurs very slowly, if at all. From the results here described it is evident that in such plasmas, at the body temperature, the action of the antithrombin must be favored to such an extent that the balance will be thrown safely to its side. Under the conditions that exist in the body in which the platelets and leucocytes remain intact and in which therefore there is no sudden increase in the content of the plasma in prothrombin and thromboplastin we can understand that the permanent fluidity of the plasma will be ensured by the protective action of the antithrombin.

THE EFFECT OF HIGH TEMPERATURES UPON THROMBIN

As pointed out by Rettger[7] solutions of thrombin made by Schmidt's method may be boiled for several minutes without losing wholly their power to cause clotting in fibrinogen-solutions. On the other hand it is very well known that if serum containing thrombin is heated to 60° the thrombic power is destroyed and when oxalated plasma is brought to the same temperature the prothrombin is destroyed or so changed that it can no longer be activated to thrombin. In the thrombin that I have prepared by acetone precipitation from saline solutions of washed fibrin I have had occasion to test at various times the degree of its thermolability, with the result that in some cases it has been destroyed apparently at relatively low temporatures, while in other cases even boiling has not removed entirely its characteristic action. Examination of these results has shown that the effect of high temperatures on the thrombin is greatly influenced by the character and amount of the salts present in solution.

Sodium chloride has a marked influence in this respect as is shown by the following experiments. A specimen of dry thrombin was dissolved in distilled water to make a 0.1 per cent solution. This solution was divided into three parts: A, con-

[7] Rettger: American Journal of Physiology, 1909, xxiv, 406.

taining no salt; B, containing sodium chloride to a strength of 0.5 to 1 per cent, and C, containing sodium chloride to a strength of 1 to 1.5 per cent. The three specimens were placed in a water-bath and heated to 60°C. for two minutes. Their action was then tested upon a freshly-prepared solution of fibrinogen in comparison with the same solutions unheated.

Fibrinogen Solution, 10 drops + Thrombin, Solution A, unheated, 2 drops gave clot in 4 minutes.

Fibrinogen Solution, 10 drops + Thrombin, Solution A, heated, 2 drops gave clot in 20 minutes.

Fibrinogen Solution, 10 drops + Thrombin, Solution B, unheated, 2 drops gave clot in 4 minutes.

Fibrinogen Solution, 10 drops + Thrombin, Solution B, heated, 2 drops gave clot in 60 minutes.

Fibrinogen Solution, 10 drops + Thrombin, Solution C, unheated, 2 drops gave clot in 4 minutes.

Fibrinogen Solution, 10 drops + Thrombin, Solution C, heated, 2 drops gave clot in 95 minutes.

The same solutions were than heated to boiling over the flame for a minute and again tested upon the solution of fibrinogen.

Fibrinogen Solution, 10 drops + Thrombin, Solution A, unheated 4 drops gave clot in 3 minutes.

Fibrinogen Solution, 10 drops + Thrombin, Solution A, boiled, 4 drops gave clot in 25 to 30 minutes.

Fibrinogen Solution, 10 drops + Thrombin, Solution B, boiled, 4 drops gave no clot in 24 hours.

Fibrinogen Solution, 10 drops + Thrombin, Solution C, boiled, 4 drops gave no clot in 24 hours.

It appears from these and similar experiments that an aqueous solution of pure thrombin free from salts is weakened but not destroyed by boiling, but that the presence of sodium chloride to a concentration of 1 per cent, more or less, renders the thrombin much more sensitive to high temperatures and effects its complete destruction at a temperature of 100°C.

No visible coagulation of the solutions was produced by the heating and the way in which the salt exerts its influence upon

the thrombin remains undetermined. It may be added that boiling aqueous solutions of thrombin, free from salts, for 5 minutes, is not sufficient to destroy their action completely. They still cause clotting of solutions of fibrinogen although the time is delayed. If the solutions are kept in a bath of boiling water for 30 minutes some remnant of activity is still maintained, for when added to solutions of fibrinogen they cause, after some time, a delicate membraneous clot.

SUMMARY

1. At temperatures approximating the body-temperature the action of antithrombin is greatly augmented. It is probable that this action is of importance in ensuring the fluidity of the circulating blood in animals like the mammals in which the content of antithrombin in the blood is small.

2. The effect of high temperatures (60°–100°C.) in weakening or destroying the action of thrombin is accelerated greatly by the presence of small amounts of neutral salts (NaCl).

CONTRIBUTIONS FROM THE ZOÖLOGICAL LABORATORY OF THE MUSEUM OF COMPARATIVE ZOÖLOGY AT HARVARD COLLEGE.
NO. 255.

THE ORIENTATION OF A HOLOTHURIAN BY LIGHT

W. J. CROZIER[1]

Received for publication October 8, 1914

I. In a paper dealing with the sensory reactions of Holothuria surinamensis, which is to appear in the Zoologischer Jahrbücher, I have shown (Crozier, 1914 [?]) that the analysis of echinoderm reactions to light given by Mast (1911, pp. 211–213) fails when applied to Aspidochirote holothurians, since (1) these animals move only with the anterior end in advance, and (2) they *orient away* from the light, yet react *negatively* to shading. It may not be out of place to publish a more detailed account of the photic orientation of another holothurian, H. captiva, which exhibits this reaction in an even more clearly defined manner.

This study was carried out at the Bermuda Biological Station for Research during the summer of 1914. For the use of the facilities of the station, my best thanks are due the Director, Prof. E. L. Mark.

II. Holothuria captiva Ludwig is a small dark-green Aspidochirote holothurian,[2] which is relatively common in certain localities in the Bermudas. It is found exclusively, during the daytime at least, clinging to the under sides of slabs of stone on rather exposed shores, just under low water mark. Individuals ranging in length from 4 to 100 mm. are obtainable during the months of June and July.[3] Experiments were made upon animals covering this range in size.

[1] Contributions from the Bermuda Biological Station for Research. No. 34.

[2] The paper on H. surinamensis contains general observations on the behavior of H. captiva.

[3] The majority of the younger specimens were found at the entrance to Hungry Bay.

The bilateral symmetry of this form is more strongly pronounced than is the case with the related H. surinamensis and H. rathbuni, though, as might be expected from the near structural affinity of all three, they show many points in common. The accentuation of physiological polarization in H. captiva is due mainly to the fact that the ventral trivium (which alone bears tube feet) is, even in very young animals, more flattened and sharply marked off from the lateral body surfaces than in the other two species; a minor factor is the greater obliquity of the oral plane, which runs obliquely ventrad and posteriad. This bilaterality is further brought out by the relatively greater rigidity of the body; lengthwise spiral twisting, common in H. surinamensis, does not occur here, and the main tendency of the body, in the absence of special stimuli, is to preserve the straightness of its horizontal axis.

The anus is situated dorsally, and when the animal is intensely stimulated, the brim about it may be elevated into a chimney-like tube, capable of being directed from side to side. This is connected with the great development of Cuvierian organs in this species, for the tube, being directed toward an irritated point on the body, controls in a general way the direction in which these organs are discharged.

In the youngest stages found (4 to 10 mm. long) the pigmentation of the body differs from that of the larger specimens, since in the very small animals the whole body is of a light green color, while in the older ones this hue persists only on the trivium, in the tentacles, and on the tips of the dorsal papillae, the dorsal and lateral surfaces becoming deep olive green. Even in the youngest specimens the tentacles are less highly colored than is the rest of the body. Darker pigmentation first becomes evident about the bases of the papillae, in animals about 8–9 mm. long; it is due, largely, to an increase in the amount of the lighter pigment, though another substance, dark brown and chemically distinct from the former, is also concerned. Some chemical characteristics of the green pigment and its possible rôle in photoreception have been treated of in the paper above referred to (Crozier, 1914 [?]), and will be further considered below.

III. Holothuria captiva ordinarily lives in dark situations. To keep it in a healthy condition for any length of time in the laboratory, it must be shaded. Prolonged exposure to even moderately bright diffused light exerts a distinctly toxic effect. It is photokinetic. The whole surface of the animal is sensitive to light. Tests made with the aid of an apparatus designed to produce small areas of light[4] gave results entirely consistent with those previously found for H. surinamensis. The tentacles reacted negatively, as shown by their partial contraction and by more or less undirected waving movements. The pedicels (when the spot-light was intense) became detached and waved about. The papillae were caused to collapse. Every point on the body-surface reacted negatively to sufficiently strong light by local in-sinking.[5] The order of sensitivity of the parts of the animal is: anterior end > posterior end > pedicels, papillae > mid-body surface.

The method of orientation to horizontal light of not too great an intensity, coming from a single source, will be apparent from an examination of the sample trails given in the text figures and in the plate. The tests, with the exception of those shown, were made by admitting sunlight, or light from a 40 c.p. tungsten filament, through a diaphram into a blackened box containing the holothurians in a flat-sided glass aquarium. Sketches of the moving animals were secured, in a dark-room, by having the aquaria mounted on short legs above a table, so that outlines of their successive positions during orientation could be conveniently traced on paper placed beneath them. The photographs were made with the holothurians exposed to two sources

[4] This apparatus, modified from one devised by Dr. B. M. Patten, consists essentially of a small pocket electric flash-light with a tungsten filament mounted, in place of the ocular, on a microscope tube provided with a low power objective (A* or "3"). Diaphrams are readily adjusted between the electric bulb and the objective, and if necessary within the lens-system, in such a way as to give beams of light of any desired cross-section and devoid of halos. The instrument as thus constructed is self contained and convenient to handle.

[5] There is no question of a heat effect being involved, since I have found that Holothuria is not equipped with anything which might properly be termed a temperature sense.

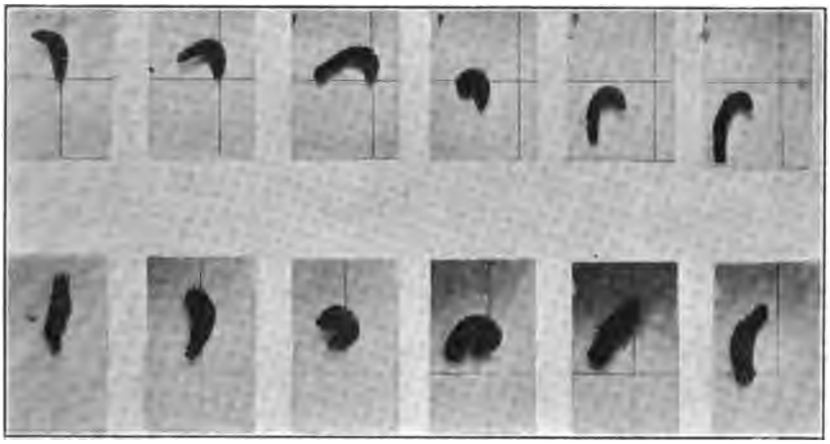
of light, diffuse daylight and more intense sunlight reflected horizontally by a mirror. The movements of orientation are sufficiently slow to permit the use of an ordinary "kodak," clamped to an upright for obtaining records. No differences in the mechanism of orientation were noticeable under these slightly different experimental conditions.

If H. captiva is suddenly subjected to horizontal light parallel to its long axis thrown on its anterior end, the pedicels of that region are released and the anterior half of the body is swung

PLATE I

Series A.

3:27 3:27.7 3:28 3:29 3:29.3 3:29.7



9:42 9:43 9:43.5 9:44.5 9:45.7 9:47.5

Series B.

EXPLANATION OF PLATE

Stages in the orientation of Holothuria captiva by sunlight parallel to its long axis incident upon its anterior end. In Series A moderately bright light was used, in Series B, light of lower intensity. The crossed lines shown were on a card placed beneath the aquarium. The rate of orientation can be judged by the relation of the animal to these coördinates at the times indicated opposite each picture.

sharply to one side (Plate I; and figs. 1, 2, 3). The swinging movement continues until the anterior end is turned as far away from the light as possible; the anterior end is then extended somewhat and re-attached; the posterior end is then frequently drawn forward by contraction of the anterior body muscles (Plate I,

figs. 2, 3); the turning away from the light continues until the animal is finally crawling in the direction of the beam. Its rate of locomotion then decreases, for the area exposed to the light is lessened; moreover, the sensitivity of the posterior end is less than that of the anterior one. Similar movements follow the application of light to the side of the animal (fig. 4). In every case the first movement is away from the light. It is only occasionally that anything which might, by any stretch of the imagination, be termed a trial movement appears. One such case is here recorded (fig. 2). It is readily accounted for by the tendency to persistence which echinoderm movements

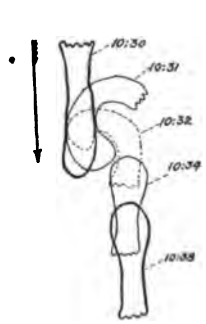


Fig. 1

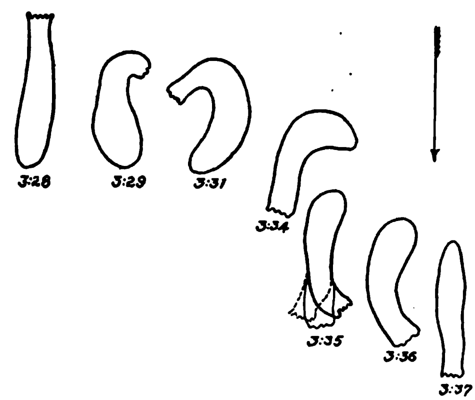


Fig. 2

Fig. 1 Stages in the orientation of H. captiva to moderately bright sunlight. Arrow shows direction of the light. The time at which each recorded observation was made is given.

Fig. 2 Conditions as in figure 1; a case which shows pseudo trial movements. The successive outlines have been shifted laterally for the sake of clearness.

in general display. The first turning movement was carried too far (3:35), and stimulation on the other side forced the anterior end to swing back; it is to be noted that these pseudo "trial movements" are not maintained.

The rapidity of orientation varies with the light intensity employed, a higher intensity giving a more rapid orienting effect (see Table 1).

Direct sunlight, or electric light of more than 200 c.m. intensity, has an almost immediate toxic action (Table 2). Animals exposed to light of these intensities were within 15 to 30 minutes caused to eviscerate through the anus or through dehiscent openings in the body wall.[6] Previous to the production of this moribund condition the animals attempted to orient, but before this reaction was completed they moved aimlessly, and later withdrew the tentacles, pedicels and papillae. After a quarter of an hour in this state very few recovered when removed to more favorable conditions.

TABLE 1

Time required for the completion of orientation by horizontal light parallel to the long axis of the animal acting on the anterior end, for individuals of different sizes; (a) with diffuse daylight, (b) with moderately bright direct sunlight. Tests were made at least an hour apart.

ANIMAL		ORIENTATION TIME, MIN.	
No.	Length, mm.	a	b
1	6	51	30
2	40	6	3
3	60	7	3
4	70	10	4
5	85	11	6

TABLE 2

Number of successive reactions to shadows, cast at 0.5 minute intervals, obtained from the anterior end of Holothuria captiva of different sizes before exhaustion; (a) in diffuse sunlight (b) after being in bright sunlight for 10 minutes.

ANIMAL		NO. OF REACTIONS	
No.	Length, mm.	a	b
1	25	3	0
2	40	7	0
3	43	3	0
4	44	4	0
5	45	4	0
6	53	18	2
7	56	8	1
8	58	16	1
9	62	9	1
10	68	19	4
11	75	19	3

[6] Under these conditions evisceration through the anus took place without the discharge of the Cuvierian organs. Taken in connection with the results of certain of my experiments, this leads me to doubt the entire correctness of Mines's explanation of the discharge of these organs as due to internal pressure. A variation of the experiment he performed on H. nigra (Mines, 1912) consisted in artificially raising the internal pressure of H. captiva by pressing on it. Cuvierian organs may thus be caused to come out through the anus at will, but their appearing and the mode and extent of their subsequent elongation are then decidedly not normal.

IV. The only conclusion permissible from the above experiments is that the behavior of Holothuria captiva toward horizontal light is a clear and definite example of negative phototropism, in the sense intended by Loeb. The animal is sensitive to light; it is one of the few echinoderms thus far investigated which possesses a strong physiological polarity as regards movement and a functionally bilateral structure; it is compelled so to adjust itself in a field of light that the effect on its opposite sides is equalized.

What is the significance of these findings in interpreting the photic reactions of starfishes, ophiuroids and sea urchins which,

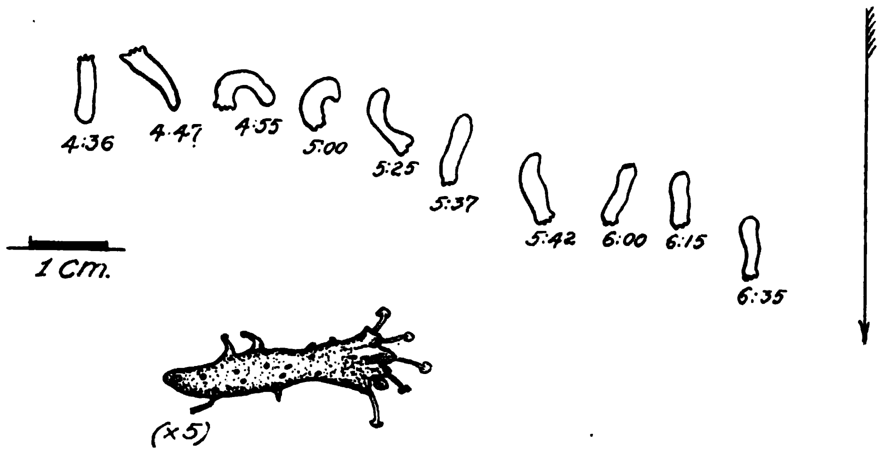


Fig. 3 Details in the orientation of an animal 6 mm. long, of which an enlarged dorsal view is given. Outlines separated laterally to avoid confusion of lines.

in contrast to H. captiva, move toward, or away from, the light without orientation? In the forms which have hitherto been studied, there is no pronounced structural or physiological prominence of an anterior end (cf. Cole 1913). They may, then, be regarded as animals which are phototropic, but in which the part which is the effective anterior changes with the altering of external (and internal) conditions. This explanation also

applies to holothurians like Thyone (Pearse 1908)[7] and (in my experiments) Cucumaria punctata. These animals are not nearly so sensitive as H. captiva, nor is their structural bilaterality so marked; in particular, functional pedicels are in Thyone found all over the body, and in Cucumaria on all five radia.

V. Holothuria captiva is likewise sensitive to shading (Table 2). It reacts negatively over its whole surface to sudden decrease in light intensity, but is not reactive to an increase of intensity. The tentacles are the most sensitive; the order of sensitivity of the parts of the animal is the same as that for the direct action of light. It is probable, therefore, that a single

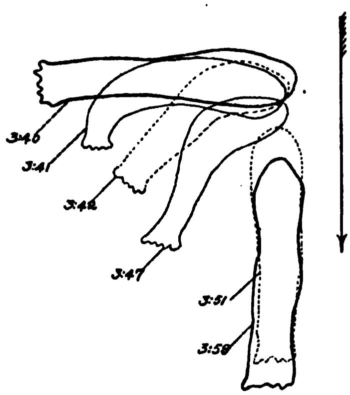


Fig. 4 Orientation by lateral light.

photoreceptive system is present, which is capable of responding to both kinds of stimulating agencies.

A detailed review of the literature on "differential sensitivity"[8] would consist almost entirely in a mere catalog of the reactions of a variety of animals to shading, or to increase in light intensity, or, rarely, to both. Such reviews may be found in the books of Mast (1914), Kafka (1914), and similar works. With the excep-

[7] Scott (1914) speaks of Thyone as being "oriented by light," but seems to use the word in a very loose sense.

[8] "Differential sensitivity" is personally preferred to Bancroft's "differential sensibility" (Bancroft, 1913); both are equivalents of "Unterschiedsempfindlichkeit."

tion of v. Uexküll's papers (1897, 1900), there are very few recorded facts of physiological significance, so far as an understanding of the mechanism involved in stimulation is concerned. It may be pointed out, however, that writers on this subject—Rawitz (1888), Nagel (1896), and particularly Hargitt (1909) and Verworn (1913)—have been troubled by the fact that a shadow, "the negation of light," can produce a positive sensory effect. The difficulty, as is clear from the discussion of Hargitt (1909), and Verworn (1913, pp. 41 et seq.), results from a too superficial view of the nature of a "stimulus." This may be seen in the following quotation from Verworn (op. cit., p. 44):

> . . . . It is altogether impracticable to define the stimulus itself in relation to the nature of the effect which the stimulus has upon the substances in the system. One can only appreciate the nature of stimulation in relation to the vital conditions and without considering the nature of the action of the stimuli on the living substance.

So far as I can discover, this statement is physiologically meaningless. It is not out of place, in this connection, to insist upon the simplicity of the view that when a sensory cell is aroused to activity the real *stimulus* is the changed physico-chemical condition within the cell, or at its surface membrane.

The integument of Holothuria is provided with pigment cells, in relation with which there terminate fibers of the radial nerve strands. Polara (1906) suggested that these were the organs of a diffuse photic sense. If we picture, possibly located in these cells, a balanced system including (1) a photosensitive material, (2) the precursors which in the normal course of metabolism go to produce it, and (3) the products of its photolytic decomposition, which may or may not be identical with its precursors, we have all the essentials of a mechanism with which to account for the photic reactions of Holothuria. The photolysis of the substance provides the stimulus for photokinetic reactions and phototropism, while the abrupt cessation of this photolytic action, or the re-institution of reconstructive reactions previously inhibited by light, is the stimulus for the shading reflex. This

view is consistent with the electrical behavior of the excised vertebrate eye under similar conditions (Einthoven and Jolly, 1908).

I was at first of the opinion that possibly the green skin pigment was itself the photosensitive material. It is fluorescent, with a bluish-green light like uranium glass, and water or alcohol extracts containing it are bleached by exposure to sunlight and air. But this conception must be modified in view of subsequent experiments. Fresh neutral or alkaline watery extracts of the skin of Holothuria contain this pigment and an abundant supply of a catalase-like enzyme. They are not bleached rapidly enough, even with the addition of hydrogen peroxide, to warrant the belief that the pigment is normally decomposed by light to any great extent. But such preparations show a notable acceleration in the evolution of oxygen from hydrogen peroxide when they are exposed to bright sunlight, which disappears on return to the shade. It is not too extreme to suggest that there is some relation between this increase in peroxide catalysis and the fact that nerve processes terminate in connection with the surface of the pigment cells. R. S. Lillie (1913) has shown that in frog leucocytes the most rapid oxidations occur at the nuclear and plasma membranes, and that these oxidations are increased by stimulation.

The fluorescent pigment therefore probably acts merely as a sensitizer. The theory of the intimate connection of this pigment with photoreception is supported, not only by the widespread occurrence of fluorescent substances in animal photosensitive organs, and the well-known influence of such materials in increasing the toxic action of light when they are injected, but also by the following observations which I have made upon cases occurring in nature:

(*a*) H. surinamensis is a nocturnal animal. In its normal habitat it comes during the night to the surface of the sandy mud in which it lives. When found, as is occasionally the case, among rocks, the surface of the body, excepting the tentacles and podia, is covered by a thin firm layer of dark silt held by a mucoid secretion. In the summer of 1914 I secured four individuals of this species which were found on the upper surface

of stones in fairly bright sunlight. They were 6 to 10 cm. in length. These specimens were devoid of greenish coloring matter, the only pigment visible being the dark brown melanoid which usually accompanies the fluorescent material. Though kept in the laboratory for some days, these animals were found to be totally insensitive to shading and only vaguely photokinetic. They did not orient to light.

(*b*) H. surinamensis and small H. captiva are sometimes found having unmistakably regenerated anterior or posterior ends.[9] Such portions are notably deficient in pigmentation and at the same time give evidence of a lower sensitivity to light and shading than that possessed by the normally pigmented tissues.

(*c*) In the three Bermudan species of Holothuria the order of increasing sensitivity to light and shading—H. rathbuni < H. surinamensis < H. captiva—is exactly that of the relative increasing amount of fluorescent green pigment in their skins.

(*d*) It is a general rule, certainly true of holothurians in other responses, that the activity of an animal is inversely proportional to its size. Comparing the number of shading reactions obtained from animals of different sizes (Table 2), it will be seen that the smaller individuals, which react, in general, more vigorously, are more quickly exhausted. The pigmentation of the very young H. captiva (about 4–6 mm. long), however, is less dense than that of older specimens, and these are much less sensitive to photic stimulation, both in the shading reflex and in photokinesis. The relative times occupied in orientation to light of standard intensity will illustrate this point (see the trails in figures 1 and 3, and Table 1).

[9] These structures in H. surinamensis can be duplicated by regeneration after experimental cutting. In nature they are probably the result of autotomous bisection. I can confirm for H. captiva the statement of Dalyell (1851) that young holothurians undergo spontaneous self division. Larger specimens of the species last mentioned do not show naturally occurring regeneration and do not regenerate in the laboratory. In this respect H. captiva resembles the majority of the genus (Torelle, 1909).

SUMMARY

1. Holothuria captiva Ludwig is photokinetic. Its whole surface is sensitive to light and to shading. It gives no reaction to an increase in light intensity. The order of sensitivity of its parts is: anterior end > posterior end > podia > mid-body surface.

2. Light above 200 c.m. intensity exerts a distinctly toxic influence.

3. Like H. surinamensis, the animal moves only with the anterior end in advance.

4. Unlike starfishes, sea-urchins, and less pronouncedly bilateral holothurians, it is oriented by light. It is negatively phototropic.

5. The photoreceptive mechanism includes the action of the green fluorescent integumentary pigment as sensitiser.

REFERENCES

BANCROFT, F. W. 1913 Heliotropism, differential sensibility, and galvanotropism in Euglena. Jour. Exp. Zoöl., vol. 15, p. 383–428, 5 figs.

COLE, L. J. 1913 Direction of locomotion in the starfish (Asterias forbesi). Jour. Exp. Zoöl., vol. 14, p. 1–32, 9 figs.

CROZIER, W. J. 1914(?) The sensory reactions of Holothuria surinamensis Ludwig. Zoöl. Jahrb., Abt. Physiol.

DALYELL, J. G. 1851 The Powers of the Creator Displayed in the Creation, etc., vol. 1, London, 286 p., 70 Pls.

EINTHOVEN, W., AND JOLLY, W. A. 1908 The form and magnitude of the electrical response of the eye to stimulation by light at various intensities. Quart. Jour. Exp. Physiol., vol. I, p. 373–416, 25 figs.

HARGITT, C. W. 1909 Further observations on the behavior of tubicolous annelids. Jour. Exp. Zoöl., vol. 7, p. 155–188.

KAFKA, G. 1914 Einführung in die Tierpsychologie: Bd. I, Die Sinne der Wirbellosen; vii + 593 p., 362 figs., Leipzig.

LILLIE, R. S. 1913 The formation of indophenol at the nuclear and plasma membranes of frog's blood corpuscles and its acceleration by induction shocks. Jour. Biol. Chem., vol. 15, p. 237–248, 1 Pl.

MAST, S. O. 1911 Light and the Behavior of Organisms. xi + 410 p., 36 figs., New York.

MINES, G. R. 1912 Note on the mechanism of discharge of the Cuvierian organs of Holothuria nigra. Quart. Jour. Micr. Sci., vol. 57, p. 301–304, 1 Pl.

NAGLE, W. A. 1896 Der Lichtsinn augenloser Thiere; 120 p., Jena.

PEARSE, A. S. 1908 Observations on the behavior of the holothurian, Thyone briareus (Les[u]eur). Biol. Bull., vol. 15, p. 259–288, 6 figs.

Polara, G. 1906 Sulla connessione delle cellule pigmentate del mesenchima cutaneo dell olothurie con i nervi cutanei e sul lore significate funzionale. Boll. Accad. Gioenia, Catania, Fasc. 88, p. 12–24, 2 figs.

Rawitz, B. 1898 Der Mantlerand der Acephalen. Jena. Zeit. f. Naturwiss. Bd. 22, p. 415–556, 1 fig., 6 Taf.

Scott, J. W. 1914 Regeneration, variation and correlation in Thyone. Amer. Nat., vol. 48, p. 280–307, 5 figs.

Torelle, E. 1909 Regeneration in Holothuria. Zool. Anz., Bd. 35, p. 15–22.

Uexküll, J. v. 1897 Der Schatten als Reiz für Centrostephanus longispinus Zeit. f. Biol., Bd. 34, p. 319–339.

1900 Die Wirkung von Licht und Schatten auf die Seeigeln. Zeit. f. Biol., Bd. 40, p. 447–476.

Verworn, M. 1913 Irritability; xii + 264 p., 62 figs. New Haven.

THE EFFECT OF RADIANT ENERGY ON THE LENS AND THE HUMORS OF THE EYE

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The object of this investigation was to determine what effect is produced on the lens and on the aqueous and vitreous humors by radiation from a quartz mercury vapor lamp, a Mazda nitrogen lamp, an electric furnace and the sun. The lenses and the aqueous and vitreous humors used were from the eyes of the pig and ox.

I

QUARTZ MERCURY VAPOR LAMP

A Cooper-Hewitt quartz mercury vapor lamp operating at 170 volts, 3.3 amperes and 2400 candle power was employed in the following experiments. Lenses were introduced into quartz tubes 1 cm. in diameter, 10 cm. in length with walls approximately 0.5 mm. in thickness. The diameter of a lens was slightly greater than the diameter of a tube so that the tubes were completely filled in an horizontal direction. A quartz tube containing lenses was filled with egg white. Similarly other tubes were filled with vitreous humor, aqueous humor, blood serum and distilled water. The tubes containing the different kinds of media were closed with rubber stoppers and placed horizontally 1 cm. beneath the surface of running water in a tank under the burner. The burner operated at 5 cm. from the surface of the water.

[1] I wish to express my thanks to Dr. E. P. Hyde, Director of Nela Research Laboratory, for the privilege of working in his laboratory and to the members of the staff for their help in this investigation.

The egg white in the quartz tube began to coagulate after 15 minutes' exposure to the light. At the end of 72 hours' exposure the egg white was a firm coagulum but the immersed lens was as transparent as at the beginning of the experiment. At the end of 120 hours' exposure a slight opacity had been produced in the part of the cortex of the lens directly exposed to the light. Parallel experiments were carried out using glass tubes but on account of the putrefaction of the material the use of the glass tubes was discontinued. Putrefaction, however, did not take place in the glass tubes for 40 or 50 hours in which time there was scarcely any precipitation of the egg white. This is in keeping with the well known fact that glass transmits very poorly the short wave lengths of the spectrum to which the coagulating property of the quartz mercury vapor lamp is due.

The vitreous humor in the quartz tube became slightly turbid after 24 hours' exposure to the light while the transparency of the lens immersed in it was unaltered. At the end of 72 hours' exposure the vitreous humor had become opaque while the transparency of the lens was still unchanged except for the part directly exposed to the light. This part was covered by a thin cloudy film. A quartz tube filled with clear vitreous humor but containing no lens was exposed to the light for 150 hours. At the end of this time there was a very slight cloudiness in the material in marked contrast to the opacity of the humor in the preceding experiment in which the lens had been immersed during the 72 hours' exposure to the light.

The aqueous humor in the quartz tube in which a lens was immersed became slightly turbid after 24 hours and opaque after 72 hours but the transparency of the immersed lens was very little affected. A quartz tube filled with aqueous humor but containing no lenses was exposed to the light for a period of 150 hours with absolutely no change in the clearness of the liquid. As in the case of the vitreous humor this clearness was in marked contrast to the opaqueness of the aqueous humor in which a lens had been immersed during the experiment.

On exposure of the quartz tube containing the lenses immersed in distilled water the liquid became turbid in 45 minutes.

After 72 hours' exposure the transparency of the lenses was scarcely affected.

Lenses immersed in blood serum contained in quartz tubes were exposed to the light. The serum was obtained by centrifugalizing defibrinated pigs' blood. After 15 hours the serum became slightly turbid and after 72 hours it was opaque. At that time there was a slight cortical opacity on the part of the lens directly exposed to the light.

II

MAZDA NITROGEN-FILLED LAMP

A 750 Watt, nitrogen-filled Mazda lamp operating at 0.6 Watt per candle power was used. By means of a plano-convex glass lens having a diameter of 4 inches and a focal distance of approximately 12 inches an image of the filament of this lamp was focused about 1 mm. below the upper surface of the materials used. The materials were similar to those on which the radiation from the quartz mercury vapor lamp was studied, viz., lenses immersed in egg white, in vitreous humor, in aqueous humor, in blood serum and in distilled water. The lenses immersed in these media were exposed to the light as described both in quartz tubes and directly in open mouthed vessels. In no case was any apparent effect produced either in the media or in the immersed lenses.

III

ELECTRIC FURNACE

The electric furnace employed was 1¾ inches in diameter, 2½ inches in depth with a heating coil of platinum wire imbedded in clay and operating at a temperature of approximately 1000°C. The materials were similar to those used in the experiments with the quartz mercury vapor lamp, viz., lenses immersed in egg white, in vitreous humor, in aqueous humor, in blood serum and in distilled water. A glass tube 5 cm. in length and 1½ cm. in diameter was filled to a depth of 3 cm. with each of these media

in turn. A float, made of thin circular cork, cut to fit the tube, with a hole in the center approximately 1 cm. in diameter was prepared. The lens was fitted into the hole in the float and held in position by means of a small piece of gauze stretched across the under side of the float and attached to its edges. The float with the attached lens was introduced into the test tube onto the surface of the medium for the experiment in question. With such an arrangement the float and the entire lens except its very upper surface were covered with the medium. The tube thus prepared was clamped into position in a tank of running water so that the material within the tube was 1 cm. below the surface of the running water outside the tube. The electric furnace heated to an intense red heat, and suspended over the mouth of the furnace, was 15 cm. from the top surface of the lens. The lens was exposed to the radiation from the furnace at this distance for 24 hours. There were indications at the end of this time of drying at the surface of the lens, but in none of the experiments was opacity produced when the furnace was 15 cm. from the material. When the furnace was operated at a distance of 5 cm., the upper surface of the lens was made opaque in 15 to 20 minutes. On placing a thermometer on the surface of the lens under these conditions there was found a rise of temperature to as much as 65°C. The obvious conclusion is that the opacity was due to the heat effect and not to the radiation.

IV

THE SUN

Apparatus similar to that used in the experiments with the electric furnace was employed. The tank of running water was replaced by a vessel containing a mixture of ice and salt. The image of the sun was focused on the lens by means of a plano-convex glass lens 4 inches in diameter and having a focal distance of about 12 inches. In this way opacity of the lenses could be produced in a few minutes, but in every case the thermometer showed that there was a great rise of temperature in

and around the lens. Presumably the opacity in this case is due to the heat effect as in the case with the electric furnace.

These experiments show that it is practically impossible to precipitate the native protein of the lens and of the aqueous and vitreous humors of the eye by means of radiant energy. In confirmation of the work of others they also show that egg white and blood serum are very easily coagulated by ultra violet radiation, while the longer wave lengths in the visible spectrum and in the infra red have no such effect. They also show that the protein of the lens, extracted by means of distilled water and by means of the aqueous and vitreous humors, is easily precipitated by the short wave lengths of the spectrum.

Dreyer and Hanssen[2] and others have shown that practically all the ordinary proteins can be precipitated with more or less ease by ultra violet radiation. In view of the experiments just described it would seem that the protein of the lens offers a conspicuous exception to the generalization in that it is practically impossible to coagulate its protein by means of ultra violet rays. In the development of cataract the lens becomes opaque and the most plausible assumption is that this opacity is due to the precipitation of its protein. As a matter of fact it is difficult to understand how opacity could be produced in any other way.

Analyses[3] of normal and cataractous human lenses show that in cataract there is a great increase over the normal in the amount of certain salts. This fact suggested that it might be possible to alter the lens protein by means of these salts so that it would be possible for radiant energy, particularly the ultra violet, to coagulate the altered protein. The result of the analyses of many hundreds of normal and cataractous lenses may be seen in the accompanying table which gives the average result as estimated for a single lens. The human cataractous lenses were obtained from different parts of the United States and from India.

[2] Dreyer and Hanssen: Comptes Rendus, 1907, cxlv, 234.
[3] Burge: Archives of Ophthalmology, 1909, xxxviii, 447.

TABLE I

	AVERAGE DRY WT. OF ONE LENS	AVERAGE WT. OF ASH OF ONE LENS	PERCENT WT. OF ASH TO DRY WT.	PERCENT OF K IN ASH	PERCENT OF Ca. IN ASH	PERCENT OF Mg. IN ASH	PERCENT OF Na. IN ASH	PERCENT OF Si. IN ASH
	mgr.	*mgr.*						
Normal adult human lens	58.99	1.40	2.30	38.80	?	?	?	0
Normal adult pig's lens	137.70	3.40	2.45	34.30	0.08	1.20	6.67	0
Embryo human lens	15.47	0.25	1.60	30.80	?	?	?	0
Cataract human lens (United States)	34.42	0.58	1.68	9.80	12.50	8.00	23.82	0
Cataract human lens (India)	92.30	1.52	1.64	5.81	6.00	1.6	25.06	3.63

This table gives the percentage composition as estimated for a single lens. It may be seen from the table that in the human cataractous lens the percentage of potassium in the ash is greatly reduced while the percentages of calcium, magnesium and sodium are greatly increased over the amounts existing in the normal lens. In cataractous lenses obtained from the United States there was no indication of silicate while those from India contained distinct amounts of the silicates of potassium, calcium and possibly of sodium. What conditions caused such a marked deposition of silicate in the cataractous Indian lenses cannot be stated.

With the results of these analyses in mind and the facts that cataract is found as a complication of diabetes and that its occurrence is frequent among glass blowers experiments were performed using the same apparatus as in the preceding experiments but with $\frac{M}{100}$ calcium chloride, $\frac{M}{100}$ dextrose, $\frac{M}{100}$ potassium chloride and very dilute solutions of magnesium chloride and of sodium silicate as media for the lenses in place of the egg white, the serum, the eye humors and distilled water employed in the previous experiments. It was found that these strengths of solutions had no effect upon the transparency of lenses immersed in them for 72 hours, or longer, in the dark or in ordinary day light. The exposure of lenses immersed in similar solutions to the radiations from the nitrogen-filled Mazda lamp, from the

electric furnace and from the sun produced no change in the transparency of the lenses, as also had been the case when egg white, serum and the eye humors were used as media. However when lenses immersed in the sugar and salt solutions were exposed to the radiation from the quartz mercury vapor lamp quite different results were obtained.

The lenses were placed in quartz tubes containing the different media. The tubes were stoppered and permitted to stand for 2 hours. At the end of this period the tubes containing the lenses and media were placed horizontally under the burner in a tank of running water 1 cm. beneath the surface of the water.

On exposure of the quartz tube containing the lenses immersed in $\frac{M}{100}$ calcium chloride the liquid became turbid in 15 minutes. The part of the lens directly exposed to the light became more and more opaque and at the end of 72 hours the half of the lens directly exposed to the light had become an opaque mass while the opposite half remained almost perfectly transparent.

Lenses that had been standing in $\frac{M}{100}$ dextrose for 2 hours were exposed to the light. The liquid in the quartz tube containing the lenses became turbid in 40 minutes. The part of the cortex of the lens on the side next to the light had become somewhat opaque in this time. This opacity after 72 hours' exposure had increased until it was about .5 mm. in depth, while the part of the lens away from the light was very slightly opaque.

On exposure of the tube containing lenses in $\frac{M}{100}$ potassium chloride the liquid became turbid after 50 minutes and after 72 hours there was a gross suspension in the liquid. The part of the cortex on the side next to the light had become at this time opaque while the opposite part of the lens remained transparent.

Lenses were placed in approximately $\frac{M}{100}$ magnesium chloride solution for 2 hours. On exposure to the light the liquid became turbid in about an hour. The part of the lens directly exposed to the light became a dense opaque mass after 72 hours' exposure

while the part not directly exposed to the light had become only slightly opaque.

Lenses were placed in a very dilute solution of sodium silicate for 2 hours. On exposure the same results were obtained as with the magnesium chloride.

These experiments show that it is possible to modify the lens protein in such a way that ultra violet radiation will precipitate it, whereas when the protein is not modified the radiation will not precipitate it.

It is possible to produce opacity of lenses by immersion in any of the above solutions but to do this it is necessary that these solutions be much stronger than those used in the experiments given above and also much stronger than ever occurs in the living animal. For example, it requires a 15 per cent potassium chloride solution to produce nuclear opacity and a 10 per cent dextrose or 1 per cent calcium chloride solution to produce cortical opacity. In trial experiments I had found that lenses immersed in a 12 per cent potassium chloride solution never developed nuclear cataract, those immersed in a 13 per cent potassium chloride solution occasionally developed it after about 12 hours and those immersed in a 15 per cent potassium chloride solution always developed this opacity after about 6 hours. The transparency of the cortex of the lenses in which nuclear opacity had developed was not noticably effected. The fact that 15 per cent potassium chloride will produce nuclear opacity in a lens suggests the possibility of a relation between the production of this type of cataract and this salt. The following experiments were devised to determine if the short wave lengths would influence the production of nuclear cataract by potassium chloride.

Lenses were introduced into a 12 per cent potassium chloride solution and were exposed to the radiation of the quartz mercury vapor burner. No opacity of the nucleus was produced after 12 hours either in the lens exposed to the radiation or in the control experiment in which the lens was not exposed. Several lenses were introduced into a 13 per cent solution of potassium chloride and were exposed to the radiation. After 12 hours the nuclei of three of the lenses became opaque while the

nuclei of the remaining lenses were transparent. In the control experiment in which lenses were immersed in the same strength of potassium chloride but not exposed to the radiation the nuclei of two of the lenses became opaque, while the nuclei of the four remaining lenses were transparent. Lenses were introduced into a 15 per cent potassium chloride solution and were exposed to the radiation. Opacity of the nuclei of all of these lenses developed after about 6 hours. The nuclei of the lenses in the control experiment became opaque in about the same time.

From these experiments it may be concluded that ultra violet radiation cannot produce nuclear opacity in lenses immersed in potassium chloride of a strength slightly less than that of a solution capable of producing nuclear opacity itself, and that ultra violet cannot hasten the production of this opacity when the lenses are in solutions which are of themselves strong enough to produce it.

In the above experiments using potassium chloride the liquid surrounding the lens became turbid after a short period. It might be objected that the ultra violet radiation had no effect on the development of nuclear opacity because the radiation did not reach the nucleus, being absorbed or scattered by the opaque liquid surrounding the lens. Experiments were carried out with the view of meeting this objection. A lens was cut in equal parts, one-half of the nucleus being left in each half of the lens. One of the halves was wedged into a quartz tube so that the cut surface was pressed firmly against the side of the tube. The tube was then filled with a 12 per cent potassium chloride solution, stoppered and placed under the burner with the nucleus exposed directly to the radiation. The exposure was continued for 48 hours. At the end of this time the nucleus showed no indication of opacity. A similar experiment was carried out using 15 per cent potassium chloride. The opacity of the nucleus of the half of the lens exposed to the radiation developed after about 6 hours. The opacity of the nucleus in the control experiment developed in about the same time.

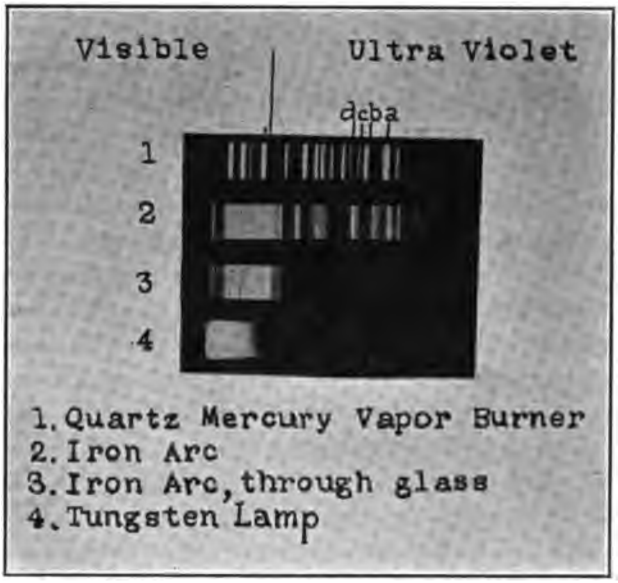
Another fact against the assumption that potassium chloride is concerned in the production of nuclear cataract is found in

the analyses of the nuclei of cataractous lenses. These nuclei did not show any increase in the content of potassium over the normal lens, but in keeping with cataractous lenses generally show a marked decrease in potassium. All this evidence would seem to indicate that potassium salts are not concerned in the production of nuclear cataract. On the other hand experiments carried out in the course of this investigation indicate that calcium, magnesium, dextrose and silicate may play an important rôle in the production of cataract in that these substances modify the lens protein in such a way that ultra violet radiation can precipitate it.

Experiments were carried out using the Fuess quartz spectograph in order to determine which wave lengths, emitted by the quartz mercury vapor lamp, caused the precipitation in the modified lens protein.

Clear fresh egg white was introduced into a quartz cell. The cell was made of two quartz disks 4 cm. in diameter and 1.2 mm. thick. These disks were separated by a ring of hard rubber 0.8 mm. thick with an inside diameter of 3.8 cm. By means of the spectrograph the spectrum from an 800 candle power quartz mercury vapor lamp was focused on the egg white in the cell. The slit of the spectrograph was 1 mm. wide and the burner was placed 3 cm. from the slit. The coagulation of the egg white began after 15 hours towards the extreme end of the ultra violet in the form of a well defined band of white coagulum corresponding in position to that of an intense band of the spectrum marked "a" on the accompanying photograph. Although "a" appears as one intense band it is in reality composed of three fused bands of wave lengths 265.2$\mu\mu$, 265.3$\mu\mu$ and 265.5$\mu\mu$, respectively. At the end of 24 hours three more bands of coagulated egg white were to be seen corresponding in position to three other bands in the spectrum, "b," "c" and "d," wave lengths 289.3$\mu\mu$, 296.3$\mu\mu$ and 302.1$\mu\mu$, respectively. From these results it would appear that the effective region in producing coagulation of the egg white is between 265$\mu\mu$ and 302$\mu\mu$ and that the most effective region is around 265$\mu\mu$ for the quartz mercury arc.

Five lenses were extracted with 0.25 per cent calcium chloride for 2 hours. At the end of this time the clear liquid was introduced into the same quartz cell and exposed in the same manner as the egg white had been. At the end of 15 hours there could be seen one delicate white band of coagulated lens protein, fairly well defined, corresponding in position to the same intense band in the ultra violet region where the egg white was first coagulated, marked "a" in the photograph. At the end of 24 hours' exposure there had appeared two more delicate white bands, more or less



Photograph of Spectograph Plate*

well defined, of coagulated lens protein. These two bands corresponded in position to the same two bands in the ultra violet region of the spectrum where the egg white was coagulated after 24 hours, marked "b" and "c" on the photograph. In place of the other band where the egg white was precipitated, marked "d" on the photograph, there was an ill-defined hazy precipitation of lens protein. The same conclusion can be drawn regarding the precipitation of modified lens protein as was drawn regarding the precipitation of egg white, viz., that the effective

*By M. Luckiesh.

region is from 265$\mu\mu$ to 302$\mu\mu$ inclusive, and that the most effective region lies around 265$\mu\mu$.[4]

It is known that cataract may be a complication of diabetes. In this disease it is also known that sugar is increased in the blood and in the body fluids and presumably in the eye media. This increase of sugar, however, is not of sufficient strength of itself to produce opacity of the lens so there must be another factor involved. The experiments cited in this paper showing the effect of ultra violet radiation on the protein of the lens modified by sugar would suggest that ultra violet radiation is the other factor. The normal lens absorbs wave lengths between 350$\mu\mu$ and 300$\mu\mu$ and transmits wave lengths longer than these. These absorbed short wave lengths do not normally produce opacity in the lens. The experiments show that very weak solutions of sugar can modify the protein of the lens so that the absorbed short wave lengths are able to precipitate the protein. The assumption that might be made in the case of diabetic cataract is that the sugar present in the humors of the eye modifies the lens protein so that the short wave lengths can bring about the precipitation. In other words, of two factors that may be involved in the production of cataract, ultra violet radiation and a modification of the protein, the latter factor is exaggerated in the production of diabetic cataract.

Glass blower's cataract, on the other hand, would seem to offer a case in which the radiant energy factor is increased. It is known that cataract occurs more frequently among glass blowers than among people generally. Crookes[5] found the radiation from molten glass in the glass blower's furnace to be very rich in red and infra red and for this reason he concludes that glass blower's cataract is due to the long wave lengths. He found that the radiation from the furnace was poor in short wave lengths. On the other hand Schanz and Stockhausen[6] found the radiation from molten glass in the glass blower's fur-

[4] These wave lengths were determined by Dr. F. M. Schultz of the University of Illinois.

[5] Crookes: Philosophical Transactions, Royal Society of London, 1914, A-509.

[6] Schanz und Stockhausen: v. Greafe Archiv f. Ophthal., 1910, lxxiii, 553.

nace to be especially rich in the region of the short wave lengths. Hence, contrary to Crookes' assumption, they conclude that glass blower's cataract is due to the short wave lengths. It seems to me that several objections could be raised to the assumptions of Schanz and Stockhausen as well as to those of Crookes. Experiments reported in this paper in which normal pig's lenses were exposed to infra red, red and ultra violet radiations without the production of opacity certainly do not bear out the conclusions of these investigators. The constitution of the normal human lens is more or less constant, the quality and quantity of radiation from the glass blower's furnace is more or less constant. If the radiation from the glass blower's furnace, whether it be infra red, red or ultra violet, be the only thing involved in the production of glass blower's cataract why is it that some glass blowers develop cataract while others do not? The fact that a large percentage of glass blowers do not develop cataract although their lenses are subjected to the same quantity and quality of radiation as in the case of those who do develop cataract would seem to imply the existence of another factor than the radiation. The fact that the normal lens protein cannot be precipitated by radiant energy while this normal protein can be so modified by chemical substances, similar to those found in cataractous lenses, that the short wave lengths in the spectrum become effective in this respect would appear to indicate as a second factor, a modification of the lens protein. The combination of the two factors named seems to offer an explanation of the fact that a relatively small percentage of glass blowers develop cataract while a large percentage although working under the same conditions do not develop it. It may be assumed that the relatively small percentage of glass blowers who develop cataract have a more or less disturbed condition of nutrition expressing itself in an increase of sugar, calcium, magnesium or some other substance which can so modify the lens protein that the short wave lengths of the spectrum are able to precipitate it. Assuming that nutritional disturbances are as frequent among workers in other occupations as among glass blowers the prevalence of cataract among glass blowers would

then be explained by the excess of the radiant energy factor. If these assumptions are true then a glass blower who has diabetes should develop cataract very rapidly. There are cases on record both among glass blowers and others where opacity of the lens once begun developed very rapidly. Unfortunately there are no data, so far as I have been able to find, which would connect these cases with the assumptions made so that the explanation suggested is based solely on the experiments reported in this paper.

The prevalence of cataract in the tropics has been noted frequently. For instance Colonel Henry Smith,[7] who kindly furnished the cataractous Indian lenses for the analyses referred to in this paper, has already performed in India more than thirty thousand operations for cataract. It may be recalled that the analyses of these lenses show the presence of a large amount of silicate. It will be remembered also that silicate is one of the substances which modifies the lens protein in such a way that the short wave lengths can precipitate it. It is known that tropical light is comparatively rich in ultra violet. A plausible explanation of the prevalence of cataract in India may be found in the combined effect of the presence of silicate in the lens and of the comparatively great amount of ultra violet radiation.

If the short wave lengths are permitted to fall on serum albumen or serum globulin, on egg albumen or egg globulin, vitellin, etc., they are absorbed and these substances sooner or later coagulate. The rapidity with which this coagulation takes place, other things being equal, depends upon the intensity of the ultra violet radiation. In view of the fact, that practically all proteins can be precipitated by means of the short wave lengths, the question naturally arises why is it almost impossible to precipitate the unmodified lens protein by similar wave lengths? The fact that it cannot be precipitated would point to an adaptive provision which needs explanation.

The lens possesses the property known as fluorescence, i.e.,

[7] Tiffany: Indian Medical Gazette, 1914, xlix, 326.

it absorbs the shorter waves and radiates this absorbed energy in the form of longer waves. It may be assumed that in this manner the lens disposes of more or less of the energy of the absorbed short waves and hence no coagulation of the protein occurs. I have found that those substances, calcium chloride, etc., which modify the lens protein in such a way that the short waves can precipitate it at the same time decrease the fluorescence of the lens. This observation lends support to the assumption that the fluorescing property of the lens protects its protein from precipitation by ultra violet radiation. However, I realize that this is merely a provisional hypothesis which must be tested by further experiments.

SUMMARY

1. Radiation from a quartz mercury vapor lamp which is sufficiently intense to coagulate egg albumen, egg globulin, vitellin, serum albumen and serum globulin in 1 hour does not coagulate the protein of the normal lens or of the vitreous or aqueous humors and hence does not affect the transparency of these structures after a continuous exposure of 100 hours.

The region of the ultra violet spectrum effective in coagulating the egg white lies between $265\mu\mu$ and $302\mu\mu$. The region most effective lies around $265\mu\mu$.

2. The lens protein can be modified by solutions of calcium chloride, magnesium chloride, sodium silicate or dextrose too weak of themselves to affect the transparency of the lens so that ultra violet radiation can precipitate the modified lens protein and hence produce opacity of the lens. The effective region in case of modification by calcium chloride is from $265\mu\mu$ to $302\mu\mu$ inclusive. The most effective region lies around $265\mu\mu$.

3. Analyses of senile cataractous human lenses show that calcium, magnesium, and in lenses from India, silicates are greatly increased in this type of cataract. The assumption is made that the accumulation of these substances modifies the lens protein in such a way that the short waves of the spectrum can precipitate the protein thus producing opacity or cataract.

4. The assumption is made in the case of diabetic cataract that the accumulation of sugar in the liquids of the body so modifies the lens protein that the short waves of the spectrum can produce opacity, hence the prevalence of cataract in this disease.

5. The above named substances which so alter the lens protein that the short waves can precipitate it at the same time decrease the fluorescence of the lens. This suggests that there may be some relation between this latter property and the great resistance of the normal lens protein to ultra violet radiation.

6. In looking for the cause of cataract it would seem that at least two factors are to be considered, the one, a modification of the lens protein, and the other, radiation of short wave lengths by which this modified protein can be coagulated.

7. Radiation from the infra red, or the visible regions of the spectrum cannot coagulate either the modified or the unmodified lens protein provided the coagulation due to heat be excluded.

CONTRIBUTIONS TO THE PHYSIOLOGY OF THE STOMACH

XVII. On the Chemical Control of the Gastric Hunger Mechanism

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The gastric hunger contractions are inhibited by mechanical and chemical stimulation of the nerve endings in the mucous membrane of the mouth, the esophagus and the stomach.[1] This insures inhibition of the hunger contractions during mastication and gastric digestion. The gastric hunger mechanism receives motor or tonic innervation via the vagi. The central connections of this tonus innervation appear to be practically isolated from all normal reflexes, while the inhibitory mechanism via the splanchnic nerves is very rapidly called into activity reflexly.[2] The foregoing facts appear to have only two alternative explanations as regards the positive control of the gastric hunger mechanism, viz.,

1. The gastric hunger contractions are due to a specific automatism (central and peripheral) primarily independent of afferent impulses as well as the conditions of the blood. Such an automatism, would, of course, vary with the physiological condition of the automatic tissues; but if this is the mechanism we cannot speak of any physiological control of the hunger apparatus, except in the way of inhibition.

2. The central and peripheral tissues concerned in the genesis of the hunger contractions may be influenced in a positive way by physiological changes in the blood. If this is the case, we might

[1] Carlson: This Journal, 1913, xxxi, p. 212; xxxii, pp. 245, 369, 389.
[2] Carlson: Ibid., 1914, xxxiv, p. 155.

expect such changes in the blood to be specially evident in the normal animal when starving.

Some facts already established seem to show that both of the above factors are to be reckoned with. In man and dog the gastric hunger contractions usually appear as soon as the stomach is empty of food, that is, before intestinal digestion and absorption of the meal is completed. Under these conditions the initiation of the hunger contractions must be due to a primary automatism, not opposed by inhibitory reflexes, rather than to any changes in the blood such as are presumably involved in starvation, for there is surely no auto-digestion of the body tissues or lack of pabulum in the body fluids, or while normal intestinal digestion and absorption is still in progress. In dogs with Pawlow stomach pouches we may also have hunger contractions in the main stomach while the Pawlow stomach is quiescent, or *vice versa*.[3] On the other hand, prolonged starvation,[4] and pancreatic diabetes,[5] which is a type of starvation, leads to increased activity of the hunger mechanism, at least up to the point where the stomach becomes directly involved in the general debility and cachexia. That increased vigor of the hunger apparatus is an after effect of a greatly accelerated metabolism is a bit of evidence pointing in the same direction.

This augmentation of the hunger contractions in starvation may be due to

1. The appearance of substances in the blood stimulating the central tonus mechanism or the peripheral hunger apparatus.
2. The absence or diminution of inhibitory substances in the blood.
3. The absence or depression of inhibitory reflexes.
4. Starvation changes in the tissues directly concerned in the hunger contraction.

If it is due to the presence of stimulating substances in the blood, it would seem that transfusion of the blood of starving

[3] Carlson, Orr and McGrath: Ibid, 1914, xxxiii, p. 119.

[4] Carlson: Ibid., 1914, xxxiii, p. 95; T. L. Patterson, experiments not yet published.

[5] Luckhardt: Ibid., 1914, xxxiii, p. 313.

animals into normal animals ought to augment the activity of the hunger mechanism, at least temporarily. We are now in position to report that this is actually the case.

THE TECHNIQUE OF THE TRANSFUSION EXPERIMENTS

Direct transfusion from the starved donor to the normal recipient by direct union of blood vessels is not feasible, because if this is done under general anesthesia, the anesthetic itself depresses the stomach, and if it is done with aid of local anesthesia only the recipient is so disturbed that the stomach is inhibited reflexly. But we found that good natured and gentle dogs used to our routine of recording the gastric hunger contractions were practically not disturbed at all by the puncture of the saphenous vein with a sharp needle and injecting 20–50 cc. fresh drawn and defibrinated blood. This technique was therefore adhered to. In the preliminary training of these dogs the animals' legs were handled in various ways (shaved, injection of salt solution, etc.), so that the animal finally paid little or no attention to the handling of the leg or the insertion of the needle into the vein. In some cases we decreased the sensitivity of the skin over the saphenous vein by the application of carbolated vaseline.

THE EFFECTS OF BLOOD FROM STARVED ANIMALS

The intravenous injection of 20–50 cc. of fresh defibrinated blood from starving dogs into normal dogs increases the gastric tonus and hunger contractions of the latter, if their stomachs are empty and moderate tonus and hunger contractions are in evidence in the recipient at the time of the injection of the blood. If the stomach of the recipient, although empty of food, is atonic and hunger contractions are completely absent at the time of the injections, the blood from starving animals has practically no action on the stomach. The stimulating action of this blood on the stomach already in moderate tonus and hunger contractions lasts from ten to thirty minutes, depending on the quantity of starved blood injected.

The above conclusion is based on 25 experiments on four gastric fistula dogs. The blood for the transfusion was drawn

from animals after five to twelve days of starvation. A typical tracing illustrating this stimulation of the hunger mechanism by small quantities of blood from starving animals is reproduced in figure 1A.

The failure of starved blood to induce tonus and hunger contractions in atonic and quiescent stomachs is probably due to the fact that by the present method of transfusion it is not possible to introduce enough starved blood to overcome the depressor or inhibitory factors responsible for the atonic and quiescent condition.

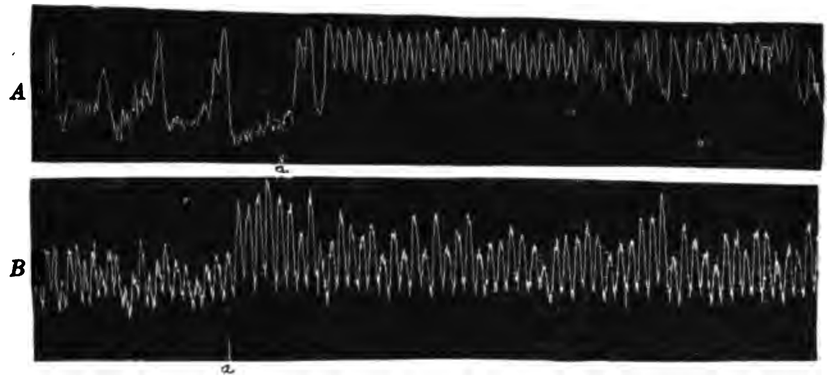


FIG. 1. TRACING FROM THE EMPTY STOMACH OF DOGS. (Reduced ⅜).

Chloroform manometer. *A*. At an intravenous injection of 35 cc. blood drawn from a dog on the eighth day of starvation. Showing stimulation of the gastric hunger apparatus, in the change from Type I to Type III hunger contractions (hunger tetanus). *B*. At an intravenous injection of 20 cc. of blood from a dog in pancreatic diabetes. Showing stimulation of the gastric hunger mechanism.

THE EFFECTS OF BLOOD FROM DIABETIC ANIMALS

Using the above technique 20–50 cc. of blood from animals in pancreatic diabetes and showing the typical diabetic polyphlagia were transfused into normal animals. The results were practically identical with those from the blood of starving animals, that is a temporary stimulation of the gastric hunger mechanism. A typical tracing showing this effect is reproduced in figure 1B.

CONTROL EXPERIMENTS

Twenty to fifty cc. of blood from normal dogs or from dogs during the height of digestion were transfused into dogs while their gastric tonus and hunger contractions were being registered. In the majority of these experiments the transfusion had no effect at all on the motor condition of the empty stomach. In a few cases it acted as a very slight and transient stimulus, but in no instance did the blood from normal animals produce the marked effects obtained from the blood of starving and of diabetic animals. Hence we conclude that the latter results are due to something in the blood of starving and of diabetic animals not present, or present in less concentration in the blood of normal animals. It is evidently not due to the transfusion of the above quantities of defibrinated blood as such. The intravenous injections of 20–50 cc. of 0.9 per cent NaCl is also without effect on the hunger mechanism.

It is well known that intravenous injections of considerable quantities of fresh defibrinated blood may cause temporary vasomotor and cardiac disturbances. Lowering of the arterial blood pressure is usually a feature of these disturbances. It is highly improbable that vaso-dilatation is a factor in the marked results produced by blood from starving and diabetic animals. The following control tests were made. One per cent peptone in 0.9 per cent NaCl was injected intravenously, and amyl nitrite was administered by inhalation. If sufficient peptone or amyl nitrite was given to affect the gastric tonus and hunger contractions, this effect was always in the direction of inhibition and paralysis. It is not clear, however, that this inhibition was due solely to the vaso-dilation, but the experiments show that a general vaso-dilation does not necessarily lead to stimulation of the gastric hunger apparatus.

THE ACTION OF THE ACETONE BODIES ON THE GASTRIC HUNGER MECHANISM

As a preliminary step in the analysis of the above stimulation of the gastric hunger mechanism by starved and diabetic blood, we have tested the action of acetone, and oxybutyric acid on the

gastric hunger contractions. We had also planned to use diacetic acid in these experiments, but we were not able to obtain this acid at that time. It is well known that prolonged starvation as well as diabetes leads to acidosis, although Mariott[6] has recently shown that there is practically no acidosis in pancreatic diabetes in dogs. It seemed possible that the acetone bodies might be the stimulating factors in the starvation and the diabetic blood. The action of the acetone bodies dissolved in Ringer's solution were tested on a number of animals with uniformly negative results. That is to say, the acetone bodies in concentrations that effect the gastric hunger apparatus at all, cause inhibition and depression. No indication of any primary or secondary stimulation by the acetone bodies could be secured. It is therefore clear that the stimulating action of starvation and diabetic blood on the hunger mechanism is not due, at least not directly, to the condition of acidosis of the blood.

THE EFFECT OF HEMORRHAGE ON THE GASTRIC HUNGER MECHANISM

It occurred to us that some of the conditions of starving might be produced temporarily by hemorrhage. It was recognized, of course, that hemorrhage also introduces factors not present, at least in moderate starvation, such as the temporary diminution of hemoglobin. Nevertheless, the results of two series of experiments with the effects of excessive hemorrhage were so striking and conclusive that they are reported here, even though we have not worked out their interpretation. The results are most conveniently stated by the following brief protocols:

October 20. Type II and III gastric hunger contractions.

October 21. Type I contractions. Gastric tonus equals 3 cm. chloroform.

October 22. Type I contractions. Gastric tonus equals 3 cm. chloroform.

October 23. Type I contractions. Gastric tonus equals 3 cm. chloroform.

[6] Marriott: Jour. of Biol. Chem., 1914, xviii, p. 507.

October 24. Type I contractions. Gastric tonus equals 2½ cm. chloroform.

October 27. 9.12 a.m. light ether anesthesia, 146 cc. blood drawn from carotid artery at 9.30 a.m. Recording of the gastric hunger contractions began 10.08 a.m. At this time the stomach was atonic and quiescent. A gradual return of gastric tonus appeared at 10.30. At 11 a.m. the gastric tonus was 5 cm. chloroform with vigorous Type III hunger contractions, and this condition persisted till the end of the experiment at 12.30.

October 28. Type I contractions. Gastric tonus equals 2½cm. chloroform.

October 29. Type I contractions. Gastric tonus equals 3 cm. chloroform.

October 30. Type I contractions. Gastric tonus equals 3 cm. chloroform.

October 31. Type I contractions. Gastric tonus equals 2½ cm. chloroform.

Control Experiment on Dog I, November 18, Ether anesthesia for 20 minutes

November 18. Type I contractions (very feeble). Gastric tonus 2 cm. chloroform.

November 19. Type I contractions (feeble). Gastric tonus 2 cm. chloroform.

November 21. Type I contractions. Gastric tonus 2 cm. chloroform.

November 25. Type I contractions. Gastric tonus 2 cm. chloroform.

November 26. Type II contractions. Gastric tonus 3½ cm. chloroform.

Dog VII. Weight 6.7 k.

October 30. Type I hunger contractions. Gastric tonus 2 cm. chloroform.

October 31. Type I hunger contractions. Gastric tonus 2 cm. chloroform.

November 3. Type I and II hunger contractions. Gastric tonus 3 cm. chloroform.

November 4. Type I hunger contractions. Gastric tonus 2 cm. chloroform.

November 5. Type I hunger contractions. Gastric tonus 2 cm. chloroform.

November 6. 9.10 a.m. 169 cc. blood withdrawn from carotid artery under light ether anesthesia. Record of gastric contractions began at 9.45. At this time the stomach was quiescent with feeble tonus. At 10 a.m. the gastric tonus began to increase. At 10.30 the gastric tonus was 9 cm. chloroform with Type III vigorous hunger contractions. This condition persisted till the end of the experiment at 11.30.

November 7. Type II and III contractions. Gastric tonus 2½–3 cm. chloroform.

November 11. Type II and III contractions. Gastric tonus 3–7 cm. chloroform.

November 12. Type I contractions. Gastric tonus 2½ cm. chloroform.

Control Experiment on Dog II. November 18, Ether anesthesia for 20 minutes

November 18. Type I and III contractions. Gastric tonus 1–4 cm. chloroform.

November 20. Type I and III contractions. Gastric tonus 2 cm. chloroform.

November 21. Type I contractions. Gastric tonus 2 cm. chloroform.

November 24. Type I contractions. Gastric tonus 2 cm. chloroform.

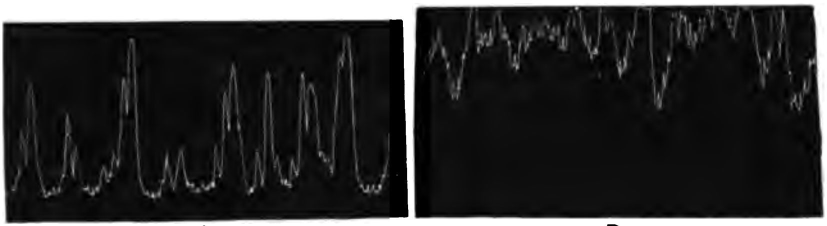
November 25. Type III contractions. Gastric tonus 3–4 cm. chloroform.

November 26. Type I and III contractions. Gastric tonus 3–6 cm. chloroform.

The reader will note that in both dogs the hemorrhage induced temporarily a greater gastric tonus and intensity of hunger contractions than typical for these dogs before the hemorrhage. This effect of the hemorrhage disappears in less than twenty-four hours. The controls show that the stimulation of the gastric tonus mechanism is due to the hemorrhage, and is not an after effect of the ether anesthesia. That they were felt as hunger contractions by the dogs was evidenced by the amount of food consumed on the hemorrhage days.

A typical tracing showing this stimulating action of hemorrhage on the hunger mechanism is reproduced in figure 2.

The following considerations might be offered not only as a possible but also as a probable explanation. The blood is, of course, the purveyor of nutritive substances to all the tissues of the body. Its chemical composition is kept remarkably constant. If now an animal is bled extensively (2–3 per cent of body weight) there is removed suddenly an enormous amount of pabulum, that is, of those *various* substances which are taken up by the *different* tissues during circulation. The organs and tissues deprived of these respective nutritive substances become hungry and give up a something (a hormone) to the circulation



A *B*

Fig. 2. (Reduced $\frac{2}{3}$).

A. Tracing showing how gastric tonus and Type I hunger contractions characteristic of Dog VII before hemorrhage. *B*. Record of gastric tonus and hunger contractions of Dog VII sixty minutes after drawing 169 cc. blood from the carotid artery. Showing the temporary stimulation of the gastric hunger mechanism as an after effect of excessive hemorrhage. (Bottom of tracing = 0 mm. pressure).

which reaching the muro-muscular apparatus of the stomach stimulates the latter to the production of the hunger contractions.

We recognize, of course, that acute hemorrhage introduces other factors. Some of them have been mentioned. The explanation offered gives a simple and reasonable picture of the mechanism involved. By acute hemorrhage we induce suddenly temporary but *acute* starvation. Probably all the tissues of the body give up this "hunger hormone." By withholding food from the animal these "hunger hormones" accumulate more slowly, depending for one thing on the state of nutrition and reserve food supply of the animal before the period of actual tissue starvation begins.

SUMMARY

1. Blood from starving animals and animals in pancreatic diabetes transfused into normal animals acts as a temporary stimulus to the gastric hunger mechanism.

2. Excessive hemorrhage is followed by a temporary augmentation of the gastric hunger contractions.

3. Prolonged starvation, pancreatic diabetes, and *possibly* excessive hemorrhage result in the increase of some substance or substances in the blood that act as stimuli to the gastric hunger mechanism.

ON THE SECRETORY INNERVATION OF THE HYPOPHYSIS

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Cushing[1] and his co-workers have recently reported experiments that seem to indicate a secretory innervation of the hypophysis. Dandy[2] has reported histological studies of the nerve running up to the gland along the arterioles. He finds that these nerves are derived from the sympathetic carotid plexus. He is unable to make any absolute differentiation between secretory and vasomotor nerves, but he is inclined to the belief that these are secretory nerves, this inclination being based on the observation that these trunks make connections with other cranial nerves, that the anterior lobe has a richer nerve supply than the posterior lobe, and that no vasomotor nerves have yet been demonstrated in the cranial cavity.

Cushing attempted to discover the presence of secretory nerves to the glands by physiologic means. If a hyperplasia or diminished activity of the gland brings about adiposity and increased sugar tolerance, an excessive activity of the gland might bring about a decreased carbohydrate tolerance and give rise to glycosuria.[3] Cushing accordingly carried out a series of experiments on rabbits consisting of a prolonged electrical stimulation of the superior cervical ganglion with the animals under anesthesia. He found that a glycosuria invariably followed such a procedure, and he therefore concluded that "*the pituitary body, and more particularly its posterior lobe, plays a significant rôle in the metabo-*

[1] Weed, Cushing, and Jacobson: This Journal, 1813, xxxi, p. xiii.

[2] Dandy: Am. Jour. of Anat. 1913, xv.

[3] Borchardt: Zeitschr. f. Kl. Med. 1908, lxvi, p. 332; Goetsch, Cushing, and Jacobson: Bull. Johns Hopkins Hospital, 1911, xxii, p. 165.

lism of carbohydrates, and its action in this respect in under the control of fibers which reach the gland by way of the cervical sympathetic ganglion. Stimulation of this nervous pathway at this ganglion liberates a chemical substance which causes glycogenolysis and glycosuria independent of any possible nervous impulse reaching the glycogen-holding cells or the abdominal viscera."

Dr. Carlson suggested to us that the glycosuria obtained by Cushing may be due to other factors than the stimulation of secretory nerves to the hypophysis. In the first place prolonged anesthesia tends to induce glycosuria especially in rabbits and cats. Handling of the vagi is almost unavoidable during the operation and on stimulation of the superior cervical ganglion it is difficult to prevent escape of current to the vagus. Even partial exposing of the abdominal viscera while inserting cannulae into the ureters affects the normal state of the animal. The animal under stimulation is therefore far from being under a normal physiological state.

At the suggestion of Dr. Carlson we have repeated Cushing's work with various modifications. Our results seem conclusive; but it must be understood that the work deals with but one method of approaching the general problem, namely, that of the carbohydrate tolerance or rather glycosuria. It leaves open all other possible avenues of approach that might definitely prove or disprove a secretory innervation of the hypophysis.

EXPERIMENTAL PROCEDURE

Rabbits, cats and dogs were used in these experiments. Both cervical sympathetic trunks were isolated from the vagi, with as little handling of the latter as possible, and then cut, leaving a silk thread attached to the central end of each. This operation was, of course, carried out under light anesthesia and aseptically. The neck of the animal was wrapped carefully, but the wound was left open so that after the animal has been given ample time (8–24 hours) to recuperate and the post operative urine tested for sugar the two nerve trunks could be stimulated while the animal was conscious and normal. The dilatation of the pupils

was indicative of the activity of the nerves. This procedure permits stimulation of the possible nervous pathway to the gland similar essentially to that carried out by Cushing, while the animal is practically in a normal condition. After a continuous stimulation for a number of hours (1–3) the animal was put into a metabolism cage and the voided urine tested for sugar.

It was planned further that, if stimulation of the nerves under physiological conditions outlined above produced no glycosuria, two kinds of controls would be run. First, the crucial operation of Cushing would be exactly duplicated, but would not be followed by any stimulation of the superior cervical ganglia. The animal would be just allowed to lie with its tracheal cannula and the urine collected from time to time and tested for sugar. By the crucial operation is meant the isolation of the cervical sympathetic nerve from all vagus association and the subsequent isolation of the superior cervical sympathetic ganglion. The second kind of control would consist of merely inserting a tracheal cannula and then letting the animal lie undisturbed without any nerve handling or without their stimulation. Here, too, the urine would be tested from time to time for sugar. These two kinds of controls would determine in the first place the combined effects of operation and prolonged anesthesia, and in the second place the effects of mere anesthesia.

Next it was planned to ascertain if the gylcosuria obtained by Cushing was in any way facilitated by reflex vagus influence as a result of the handling or stimulation in the operation. For this purpose it was decided to section the splanchnics aseptically immediately below the diaphragm and, after the animal has recuperated sufficiently, to repeat the crucial operation. The cutting of the splanchnics would, of course, obviate later any possible reflex vagus influence on the adrenals or the liver.

Finally, if it were found that no glycosuria occurred as a result of stimulating the cervical sympathetic nerves under physiological conditions, it was planned to determine whether or not such stimulation was capable of producing a measurable hyperglycemia. For this purpose samples of blood would be drawn from the conscious animal at definite intervals before, during and

after stimulation of the nerves, and the sugar content then determined.

The administration of ether to rabbits and cats was carried out by means of a large bell jar so as to obviate excessive struggling and emotional glycosuria. Fifty to one hundred cc. of water were given by a stomach tube to insure the getting of urine. For the stimulation a tetanizing current was used. A tetanometer giving 3–8 stimuli per second was always placed in the circuit to prevent a too rapid bombardment of stimuli. During the stimulation the animal was kept in an oilcloth bag in such a way that merely the head and neck were free. This served to prevent struggling as well as a possible loss of urine. For the crucial operation a cannula was inserted into the urethra. An incision into the abdominal cavity very close to the symphysis oss. pubis was made just large enough to permit pulling out of the urinary bladder. This obviated any exposure or handling of the abdominal viscera which is quite unavoidable if cannulae are inserted into the ureters. The qualitative and quantitative determinations of the urine sugar were made by the Fehling's and Benedict's methods respectively. The blood sugar content was determined by the Rona-Michaelis method.

RESULTS

The following results were obtained from a series of experiments carried out on 15 cats, 4 rabbits, and 3 dogs.

Crucial Operation

This comprises a series of 6 experiments carried out on 4 cats, 1 rabbit and 1 dog. Cannulae were inserted into the trachea and urethra respectively. Each sympathetic nerve was traced up to the superior sympathetic cervical ganglion, which was separated from the vagus ganglion lying in the same sheath. Then the ganglia were stimulated alternately at intervals of one minute. One typical experiment is given in table I.

It is seen that here there was sugar in the urine at the end of 1½ hours anesthesia and operation, even before stimulation of

TABLE I

Cat 5

SAMPLES OF URINE COLLECTED	VOLUME	SUGAR	
	cc.	per cent	gram
Preoperative urine	105.0	0	0
From bladder at beginning of operation	3.2	0	0
During operation and before stimulation; End of ½ hour anesthesia	2.5	8.3	0.210
End of 2½ hours anesthesia; End of one hour stimulation	2.0	8.5	0.170
End of 3 hours anesthesia; End of 1½ hours stimulation	1.5	6.0	0.090
End of 4 hours anesthesia; No stimulation	1.0	4.3	0.043
End of 5 hours anesthesia; No stimulation	1.5	2.7	0.030

the superior cervical ganglia and the percentage of sugar bears no relation to the stimulation. This is true of all the experiments in this group.

Physiological nerve stimulation with urine sugar tests

This group includes 10 experiments carried out on 3 rabbits and 6 cats. Both cervical sympathetic nerves were cut aseptically and stimulated after the animal had recovered from the operation and the post-operative urine had been tested for and found free from sugar. The animals were kept in the bag and held on the lap so that they lay comfortably and quietly while the nerves were being stimulated. The results are summarized in table II.

This table shows clearly that in all animals except Cat No. 1 there was never a trace of sugar in the urine resulting from stimulating both cervical sympathetic nerves while the animals were conscious and normal. Even in that exception (Cat No. 1) it cannot be stated with certainty whether the slight amount of sugar was due to the effects of the operation or to the stimulation, for no post-operative urine was obtained to be tested.

TABLE II

ANIMALS AND NUMBER	PREOPERATIVE URINE 24 HR. SAMPLE		POST OPERATIVE URINE 24 HR. SAMPLE		POST STIMULATION URINE 24 HR. SAMPLE		DURATION OF STIMULATION
	Volume urine	Sugar	Volume	Sugar	Volume	Sugar	
	cc.	*per cent*	*cc.*	*per cent*	*cc.*	*per cent*	*hours*
Rabbit No. 1 (right sympathetic nerve)	10	0	11	0	11	0	2
Rabbit No. 1 (left sympathetic nerve)	70	0	50	0	25	0	1¾
Rabbit No. 2 (both nerves)	100	0	70	0.47	80	0	1½
Rabbit No. 3 (both nerves)	40	0	20	0	70	0	1¼
Cat No. 1 (both nerves)	85	0	none		60	0.96	1
Cat No. 2 (both nerves)	100	0	52	1.7	33	0	1
Cat No. 3 (both nerves)	none		33	0	50	0	1
Cat No. 4 (both nerves)	115	0	?	0	30	0	1¼
Cat No. 5 (both nerves)	50	0	105	0	105	0	1½
Cat No. 6 (both nerves)	70	0	180	0	160	0	1¼

Physiological nerve stimulation with determinations of the blood sugar[4]

Having failed to produce glycosuria by stimulating the cervical sympathetic nerves under physiological conditions, we next set out to determine whether this stimulation could at least produce a hyperglycemia which was either too fleeting or too slight for sugar to enter the urine. For this purpose we drew samples of blood from the tails of two cats before, during and after stimulation of the nerves. But we found that it was next to impossible to keep the cats from getting excited and struggling while the blood is being drawn. The excitement, of course, produces an emotional hyperglycemia, as shown by Cannon and his co-workers. To obviate this we inserted carotid cannulae into two cats so that blood could be drawn very quickly without disturbing the animal. We repeated this test on two dogs. The dogs did not struggle at all while the samples of blood were being drawn from the tail or from a leg vein. The results are given in table III.

[4] The blood sugar determinations were made by Mr. H. Ginsburg.

TABLE III

	DOG NO. 2	DOG NO. 3	CAT NO. 14	CAT NO. 15
Per cent of sugar in the blood before stimulation of both sympathetic nerves......	0.11	0.13	0.194	0.181
Per cent of sugar in the blood at the end of 1 hour's stimulation....................	0.10	0.10	0.143	0.180
Per cent of sugar in the blood at the end of 2 hours' stimulation....................	0.13	0.12		

It is quite clear from the foregoing table that there is *not even a perceptible hyperglycemia resulting from the stimulation of the cervical sympathetic nerves under physiological conditions.* The high sugar content observed in Cats Nos. 12 and 13 were undoubtedly emotional hyperglycemia, even though the animals did not seem frightened or restless.

Controls

This group comprises a series of experiments carried out on 5 cats and 16 dogs. They were intended to determine the effects of the operation, vagus handling, and prolonged anesthesia without any stimulation of the cervical sympathetic nerves or ganglia. In Cat No. 6 the crucial operation was exactly duplicated, except that the operation was not followed by stimulation. In the rest neither the nerves nor the ganglia were even exposed, but the animals were merely allowed to lie under anesthesia with tracheal and urethral cannulae, the urine being collected at definite intervals and tested for sugar.

The urine was collected from the excised bladders of 16 dogs that had been kept under ether anesthesia for two to four hours each. Upon an analysis the urine of 7 of these 16 dogs was found to contain sugar, the percentage ranging between 1.2 per cent and 1.6 per cent. In other words, 43.7 per cent of these dogs had a glycosuria resulting from prolonged ether anesthesia alone.

All of the cats showed glycosuria at the end of one to one and one-half hours ether anesthesia. In 4 of the 5 cats of this series the nerves or ganglia were not even exposed, and yet the sugar in the urine ran a similar curve. It began to rise gradually at

the end of one to one and one-half hours anesthesia, reached its maximum at the end of three to four hours, and then gradually declined. The fact that in the dog group this was by far not so pronounced points to the fact that this is largely a question of the degree of susceptibility of the species, the dog being more resistant than the cat and the rabbit.[5]

Possible vagus influence on the glycosuria in the crucial operations

The object of the experiments in this group was to determine whether the glycosuria following the stimulation of the superior cervical ganglia was due solely to anesthetic effects or to the combined effects of anesthesia and vagus exposure and handling while isolating the ganglia. For this purpose the splanchnics were cut aseptically in two cats immediately below the diaphragm so as to obviate later any possible reflex vagus influence on glycosuria production. Both animals were then given a few days to recover fully, and then the crucial operations were carried out in the ordinary way with the results shown in table IV.

It is evident from table IV that the vagus does not in any noticeable degree partake in the glycosuria production during the crucial operation. For here the glycogen-holding viscera were cut off from all reflex vagus influence, and yet the sugar ran the ordinary curve. Again this table shows clearly that sugar appeared in the urine before any stimulation of the cervical sympathetic system.

SUMMARY

1. Stimulation of the cervical sympathetic nerves while the animal is conscious and under physiological conditions does not produce hyperglycemia, glycosuria, or diuresis. This is true for dogs, cats and rabbits.

2. In all cases where the animal is subjected to a crucial operation and prolonged ether anesthesia, glycosuria appears whether or not the superior cervical ganglia are stimulated. A similar

[5] Carlson and Ryan: This Journal, 1908, xxi, p. 301.

TABLE IV

Cat No. 11

SAMPLES OF URINE COLLECTED	VOLUME	SUGAR		VARIATIONS IN MANIPULATION
	cc.	per cent	gram	
From bladder at beginning of operation	10	0	0	Preoperative
End of ½ hour anesthesia; during crucial operation	1½	0	0	During operation 1 hour.
End of 1 hour anesthesia; during crucial operation	1	2.8	0.028	
End of 1½ hours' anesthesia; no operation and no stimulation	1½	4.3	0.64	No operation or stimulation.
End of 2 hours anesthesia; End of ½ hour ganglia stimulation	2	4.75	0.095	
End of 2½ hours anesthesia; End of 1 hour stimulation	2	6.5	0.130	During 2½ hours of ganglia stimulation.
End of 3 hours anesthesia; End of 1½ hours stimulation	2	4.06	0.092	
End of 3½ hours anesthesia; End of 2 hours stimulation	1¾	4.7	0.082	
End of 4 hours anesthesia; End of 2½ hours stimulation	1	2.8	0.028	
End of 4½ hours anesthesia; No stimulation	1½	2.8	0.035	No stimulation
End of 5 hours anesthesia; No stimulation	3	1.6	0.048	

glycosuria occurred when the animal was subjected to mere prolonged anesthesia without even exposing the nerves or ganglia.

3. Subjection to prolonged anesthesia after exclusion of all possible downward impulses to the abdominal viscera through vagus reflexes, with or without stimulation of the ganglia, led to a similar glycosuria.

4. The glycosuria of prolonged ether anesthesia runs a uniform course, the amount of sugar gradually rising to a maximum and then gradually declining. This course is not influenced by the stimulation of the cervical sympathetic nerves, or the superior cervical ganglia.

Our results go to show that the presence of secretory nerves governing the activity of the hypophysis cannot be demonstrated by the glycosuria or hyperglycemia methods. The glycosuria resulting from the stimulation of the superior cervical ganglia is undoubtedly due to the effects of prolonged anesthesia and not to an excessive activity of the hypophysis caused by stimulation of a secretory nervous pathway to the gland. As stated previously, this work concerns itself solely with but this single method, but it leaves open all other possible methods that might be attempted definitely to prove or disprove a secretory innervation of the hypophysis.

The nerve fibers to the hypophysis described by Dandy may be vasomotor nerve fibers. The fact that no vasomotor nerves have been conclusively demonstrated in the cranial cavity does not prove that such nerves are absent from a complex gland like the hypophysis. And the fact that the anterior lobe has a vastly richer nerve supply than the posterior lobe does not in the least prove that these nerves are of a secretory nature. In fact, Cushing favors the view that the posterior lobe is mainly concerned in the internal secretion playing a rôle in the metabolism of carbohydrates.

We wish to express our gratitude to Dr. Carlson for his valuable advice and kind supervision of this work.

STUDIES OF AUTONOMIC THRESHOLDS

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I. SMOOTH MUSCLE INNERVATED BY THE CERVICAL SYMPATHETIC

It is usually stated that similar tissues in different parts of the body vary with respect to the strength of stimulus required to arouse them to activity. Also it is generally admitted that in electrical stimulation of nerves, some require strong, others weak stimuli to excite activity in the tissues innervated by them. Hitherto the methods used for studying quantitatively the strength of stimulus have been unreliable. The advent of the Martin system of quantitative stimulation, permitted a greater degree of accuracy to be attained.

The present investigation was undertaken for the purpose of ascertaining whether the strength of stimulus necessary to provoke response of tissue supplied by autonomic fibers is essentially the same for all fibers, or whether it varied with respect to the tissue supplied, i.e., muscular or glandular. The Martin method[1] of quantitative stimulation has been used throughout the research. By this method it is possible to compare thresholds within the autonomic system itself as well as thresholds of the autonomic system with those of ordinary somatic nerves.

Inasmuch as no reliable method of quantitative study by means of faradic stimuli has been available before, it is not surprising to find a paucity of references to such studies in the literature. Occasionally a statement is seen that the secondary coil was in a certain position for one stimulation and in another position for a second stimulation. A brief consideration of the

[1] Martin: The Measurement of Induction Shocks, New York, 1912.

secondary positions noted, and of the fact that the primary current is practically neglected convinces one that the quantitative comparisons must be far from precise.

In this study the cervical sympathetic in the neck was used, and responses noted in three smooth muscle structures which it is known to innervate; pupil, nictitating membrane, and the blood-vessels of the nasal mucous membrane. Movements of the pupil and nictitating membrane were observed directly. Constriction of the nasal vessels was observed by a method modified from that described by Tschalussow.[2] This observer really made a plethysmograph of the nasal cavity. He packed the posterior nares with material soaked in vaseline, thus closing the nasal cavity posteriorly; one of the anterior nares was packed with the same material, and in the other was placed a hollow glass tube. This tube was connected by rubber tubing to recording apparatus. Tschalussow found the method eminently satisfactory for the problem which he was investigating. In the present research it became necessary in detecting thresholds to have a perfectly smooth line recorded in order to observe the slightest departure from it in the way of vasoconstriction. Tschalussow's technique was not so well adapted here because it gave an irregular base line instead of a smooth line. The irregularities in the line were due to respiratory movements of the soft palate and to swallowing movements.

Instead of applying a simple packing in the posterior nares, a brass rod about one-eighth of an inch in diameter was used (fig. 1). One end of the rod was bent almost completely over on itself and terminated in two rather sharp prongs directed backward. A wad of cotton was tied over these prongs and then soaked with vaseline. An adjustable crossbar was fastened on the straight end of the rod. After the animal was anesthetized the vaselined cotton on the prongs was inserted beyond the soft palate and pulled forward by traction on the rod until progress was stopped by plugging the posterior nares. Then the crossbar was adjusted to fit snugly behind the canine teeth. Next

[2] Tschalussow, M. A.: Archiv fur die gesammte Physiologie, cli, 1913, p. 524.

the mouth was closed and tied shut by heavy twine. This twine passed in front of the upper canine teeth, then back of the crossbar and around the animal's lower jaw. In this way the packing was securely fastened; and at the same time any variation in size of the nasal cavity through respiratory or swallowing movements acting on the soft palate was effectively shut off. Next one of the anterior nares was packed with vaselined cotton; then a glass tube was placed in the other and packed air-tight with vaselined cotton. The incisor foramina were closed with plasticine. The glass tube was then tied to the brass rod which extended about three inches beyond the animal's mouth. In this way the apparatus was firmly anchored. The glass tube was then connected by rubber tubing with a sensitive tambour. Constriction of the nasal vessels was indicated by a fall in the writing lever, vaso-dilatation was indicated by a rise in the

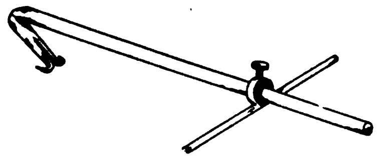

Fig. 1. Apparatus Used to Close the Posterior Nares

lever. The idea of Tschalussow to use the nasal cavity as a plethysmographic indicator is of great value. It is undoubtedly the most sensitive indicator of vaso-motor response that we possess.

Cats were used throughout this research. Some of them were anesthetized with urethane; others were anesthetized at first with ether and then rapidly decerebrated. One cervical sympathetic was cut and glass shielded electrodes[3] were placed on the nerve. These were connected with an induction coil calibrated according to Martin.[4]

Table 1 shows the results of determining thresholds and gives some idea of the range in different animals. The average thresh-

[3] Sherrington: Journal of Physiology, 1909, xxxviii, p. 382.
[4] Martin: Loc. cit.

old value for the pupils is seen to be 5.73 Z units of Martin; next in order is the nictitating membrane with an average of 6.34 Z units and then the nasal vasoconstrictors with an average of 7.89 Z units. It is readily seen that all three are of the same order of magnitude and the average of the three averages might be taken as approximately the threshold of the cervical sympathetic in the neck. It is 6.65 Z units. The corresponding β units of Martin were not estimated in all cases, but the average of those that were estimated fell in the same order. The averages were for the pupil 3.32 β units, for the nictitating membrane

TABLE I

PUPIL		NICTITATING MEMBRANE		NASAL VESSELS	
Z	β	Z	β	Z	β
1.68		0.97		2.10	
1.87		1.87		3.80	
2.00		2.00		4.00	
2.50		2.50		4.20	
2.75		2.83		5.00	
3.92		3.72		5.00	
4.00		4.00		7.40	
5.00		4.00		9.00	
8.40		10.30		9.60	
10.30		11.00		11.00	
11.00		15.40		15.40	
15.40		17.60		18.20	
Average....5.73	3.32	6.34	3.68	7.89	4.58

3.68 β units, and for the nasal vasoconstrictors 4.58 β units. The average ratio of β to Z in these experiments was 0.58. This is of particular interest because it is almost identical with the ratio (0.57) obtained by E. L. Porter[5] in a large number of experiments on peripheral nerves. In view of this evidence it is thought justifiable to calculate β units for the whole series on the basis of this ratio.

The threshold of the nasal vessels was estimated for the vasoconstrictors since these fibers have been shown[6] to be present in the cervical sympathetic.

[5] E. L. Porter: This Journal, 1912–13, xxxi, p. 149.
[6] M. A. Tschalussow: Loc. cit., p. 523.

Urethanized and decerebrate animals were strikingly similar in the responses obtained, but if ether was used it was found that a greatly increased strength of stimulus was necessary to reach the threshold. Thus observation 19 on March 24 in an urethanized animal gave a threshold of 4 Z units for the nasal vessels; on March 25, observation 25, the same threshold in an etherized animal was 26.5 Z units; and on April 3, in observation 21 (decerebrate cat) the threshold was 4.2 Z units. The thresholds for pupils and nictitating membrane were correspondingly high in etherized animals.

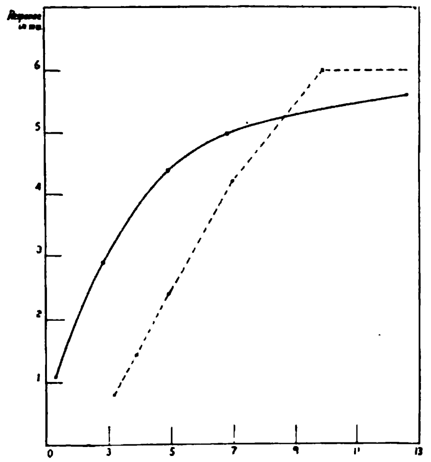

Fig. 2. Continuous curve shows response of nasal vessels plotted against stimulus strength (1.5 to 13 times the threshold). Dotted curve shows blood pressure changes in mm. of Hg plotted against strength of stimulus increasing from threshold (W. T. Porter).

Since a graphic record was kept of the response of the nasal vessels it was possible after the experiment to measure the degree of response and relate it to the strength of stimulus used to produce it. Figure 2 shows a curve in which strength of stimulus is plotted against degree of response. The abscissae represent the strength in terms of the threshold. Thus a stimulus whose strength is three times the threshold is called three. The or-

dinates represent the response in millimeters of change from the base line. No attempt was made to estimate the varying size of the nasal cavities so that the figures represent approximation only. Notwithstanding this fact there is a striking parallelism between this curve and the one plotted below with the interrupted line. This is a curve plotted by W. T. Porter[7] showing the response of the vasomotor center to increased strengths of stimuli. In the light of Dr. Porter's investigation, the nervous elements involved are conductors merely, and the bloodpressure change is an approximate index of contraction of bloodvessels. The two curves show a striking similarity of response of bloodvessels to increasing strengths of stimuli.

Another interesting fact brought out in this research is the close correspondence between the response of a peripheral autonomic fiber and the response in a reflex arc as found by E. L. Porter.[8] He found the average strength required for the flexion reflex to be 5.2 Z units. Combining the averages for pupil, nictitating membrane and nasal vessels in this investigation gives an average of 6.65 Z units for the cervical sympathetic. In the same investigation Porter showed an average of 2.3 Z units necessary to provoke response by stimulation of a peripheral nerve. The average β units for the cervical sympathetic was found to be 3.8. Porter found the average β units for the flexion reflex to be 2.7 and for peripheral stimulation 1.4. As previously stated he found the ratio of β to Z was 0.57; in this investigation it was 0.58.

It is very suggestive that the sympathetic in the neck should show a threshold equal practically to that of the flexion reflex. It is usually accepted as a fact that each synapse increases the resistance to the passage of an impulse, although the only quantitative evidence we have for that is shown by E. L. Porter's work. In his investigation as well as in this, at least one synapse was involved. Stimulation of the cervical sympathetic beyond the superior cervical ganglion seems to offer a means of determining whether the resistance resides in the synapse or in the

[7] W. T. Porter: This Journal, 1910–11, xvii, p. 278.
[8] E. L. Porter: Loc. cit.

peripheral organ. Some experiments which will be described in a subsequent paper are already planned for an answer to this question.

Another question suggested in this research is whether the three sets of fibers supplying nasal vessels, nictitating membrane and pupil really vary in their thresholds or whether the variation in thresholds is due to some peculiarity of the tissues in which the fibers end. This is a question which is pertinent to threshold values of any nerve and which must be determined by action current measurements. The point of practical importance to the physiologist is to know how much stimulus is necessary to apply to a nerve in order that it be physiological or at least comparable to normal stimulation.

SUMMARY

1. The application of quantitative stimulation to the autonomic system is described.

2. The threshold for contraction of an intrinsic smooth muscle of the eye (pupil) stimulated through the cervical sympathetic is 5.73 Z units, 3.32 β units.

3. The threshold for contraction of an extrinsic smooth muscle of the eye (retractor muscle of the nictitating membrane) stimulated through the cervical sympathetic is 6.34 Z units, 3.68 β units.

4. The threshold for contraction of smooth muscle of the nasal vessels (vasoconstriction) stimulated through the cervical sympathetic is 7.89 Z units, 4.58 β units.

5. The average threshold for the foregoing functions of the cervical sympathetic is 6.65 Z units, 3.86 β units.

6. The response to increasing strength of stimulus occurs in the order named, pupil, nictitating membrane, nasal vessels.

7. The degree of response of the nasal vessels is within limits directly proportional to the strength of stimulus.

The author wishes to express appreciation to Dr. E. G. Martin at whose suggestion this problem was undertaken and under whose supervision it was made possible.

THE CAROTID BLOODFLOW IN RELATION TO THE INTRA-ABDOMINAL PRESSURE

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Measurements of the bloodflow in the femoral vein have shown very clearly that the influx from the posterior extremities may be greatly altered by subjecting the intra-abdominal bloodvessels to different degrees of pressure.[1] Thus, it was readily possible to produce marked reductions in the femoral flow by moderately inflating the peritoneal cavity with air or by exerting gentle pressure upon the external surface of the abdomen. Quite similar results followed rather strong contractions of the diaphragm, induced by stimulation of the phrenic nerves.[2] No doubt, the descent of this membrane acts in the same way as the procedures just mentioned, all of them tending to lessen the vascularity of the abdominal viscera by first causing a greater venous discharge, which is followed later on by a reduction in the quantity of the blood normally entering the abdomen and posterior part of the body. As the venous channels are more readily compressible than the arterial, this reduction is ushered in by phenomena of venous hyperaemia and stagnation, while an arterial obstruction results only with high degrees of intra-abdominal pressure. Eventually these procedures lead to circulatory disturbances which are very similar to those resulting from occlusion of the abdominal aorta.[3]

[1] See Burton-Opitz, Pflüger's Archiv, cxxi, 1908, 156.

[2] See Burton-Opitz, American Journal of Physiology, vii, 1902, 435.

[3] Consult Sollmann and Pilcher, American Journal of Physiology, xxxi, 1912, 193.

In accordance with the results just mentioned it may justly be assumed that these impediments to the circulation through the posterior part of the body exert a profound influence upon the distribution of the blood as a whole by greatly augmenting the flow through the vessels of the head. To prove this contention, measurements of the bloodflow through the carotid and jugular systems of dogs were undertaken while different degrees of intra-abdominal pressure were established every now and then by inflation of the peritoneal cavity. The latter end was attained with the help of an ordinary trocar connected with an air pump, the degrees of pressure obtained being indicated by a manometer inserted in this circuit. The registration of the quantities of blood was accomplished by means of a stromuhr[4] inserted either in the left carotid artery or in the right external jugular vein. A membrane manometer connected with the central cannula of this instrument, served to indicate the bloodpressure.

Three experiments in all were made for the purpose of recording the changes occurring under these conditions in the carotid arteries, but as perfectly harmonious results were obtained, I may be permitted to shorten this discussion materially by considering in detail only a limited number of the phases of experiment 3, performed on February 27, 1914. The values of the bloodflow recorded during these periods, as well as other essential details, are contained in the accompanying table 1.

To begin with the bloodflow amounted on the average to 2.3 cc. in a second, while the pressure equalled about 108.5 mm. Hg Shortly after the beginning of phase 8 the abdominal cavity was inflated gradually, until the pressure therein rose to 20 mm. Hg, the inflation being continued during a period of about 70 seconds.

A comparison of the values of the bloodflow shows very clearly that the quantity of blood propelled during the inflation, is very much greater than that registered previous to this period of high intra-abdominal pressure. It is also evident that the augmentation begins almost immediately and gradually becomes more

[4] The recording stromuhr described by me has been used for this purpose. Pflüger's Archiv, cxxi, 1908, 150.

TABLE I

Carotid bloodflow in relation to intra-abdominal pressure. (Experiment 3, February 27, 1914)

Dog 12.5 Kg. Stromuhr in carotid artery

PERIOD	DURATION OF PERIOD	TOTAL QUANTITY OF BLOOD	QUANTITY OF BLOOD	BLOOD PRESSURE (CAROTID)	PROCEDURE
No.	*sec.*	*cc.*	*per sec.*	*mm.* Hg	
1	8.8	22.8	2.59	105.0	
2	10.7	22 5	2.10		
3	7.2	16.1	2.23		
4	8.0	19.2	2.41		None
5	10.0	23 0	2.30	108.5	
6	8.3	20.3	2.44		
7	9.0	19.5	2.16		
Average			2.31	108.5	
8	7.7	20.5	2.66	125.0	
9	7.3	20.0	2.74	138.5	
10	6.4	20.0	3.13	146.0	
11	4.7	17.2	3.66		
12	3.5	17.2	4.91		
13	3.6	17.5	4.86		Intra-abdominal pressure raised to 20 mm. Hg
14	3.3	17.5	5.30		
15	3.7	20.5	5.54	140.0	
16	3.3	19.4	5.87		
17	3.0	17.4	5.60		
18	3.2	18.5	5.78	135.0	
19	3.2	18.2	5.68		
20	6.0	19.3	3.21	120.0	
21	7.1	17.3	2.43	92.0	None
22	8.0	17.5	2.18		
23	8.0	18.5	2.31	102.4	
Another procedure tested					
37	7.5	16.2	2.16	112.0	
38	8.0	18.5	2.31		
39	8.6	18.6	2.16		
40	5.6	18.9	3.37	121.0	
41	4.8	18.0	3.75	130.5	
42	5.0	19.5	3.90		
43	4.5	19.4	4.31		Intra-abdominal pressure raised to 25 mm. Hg
44	4.1	19.5	4.75		
45	4.2	20.0	4.76	125.0	
46	3.5	19.5	5.57		
47	3.5	20.2	5.79		
48	3.2	19.5	6.09		
49	3.4	19.2	5.63		
50	7.0	19.0	2.71	115.0	
51	8.2	20.0	2.43	90.0	
52	10.8	19.6	1.81		None
53	10.0	19.8	1.98		
54	10.5	19.2	1.82	95.5	

conspicuous in the course of the inflation, but naturally, a short time elapses before the maximal value of the flow is attained. The increase preserves in a measure a direct relationship to the degree of pressure exerted. In the experiments before us the flow is doubled with about 20 mm. of pressure and is rendered three times greater than normal by pressures ranging from 30 to 40 mm. Hg. Normal conditions are again established very shortly after the intra-abdominal pressure is permitted to return to its original low level.

This augmentation in the carotid flow is always accompanied by a rise in the general bloodpressure, the amplitude of which is in agreement with the amount of blood diverted to this particular region. It must be concluded therefore that this rise in the general pressure which is a familiar phenomenon to most of us,[5] finds its origin in the transfer of a large amount of blood from the circuits of the posterior part of the body into those of the head, forelegs and adjoining regions.

The foregoing phenomena may be rendered especially conspicuous by digital compression of the abdominal aorta below the diaphragm. It seems quite natural to suppose that this procedure possesses an influence upon the distribution of the blood which is very similar to that exerted by a high intra-abdominal pressure. While the posterior vascular channels are rendered more or less empty, the fore part of the body is made to accommodate an extra amount of blood.

A clear idea regarding the character of the changes occurring under these conditions can readily be obtained from the accompanying table II. It seems superfluous to insert a larger number of experiments, because the one here submitted fully proves the points previously emphasized. The carotid bloodflow amounted in this case to 2.24 cc. in a second, while the pressure continued at about 118.5 mm. Hg. The occlusion of the abdominal aorta raised the value of the bloodflow almost immediately to 4.68 cc. per second and the pressure to 145.6 mm. Hg,

[5] A very comprehensive study of intra-abdominal pressure has been made by H. Emerson. See: Archiv of Int. Med., vii, 1911.

these values being retained with slight fluctuations throughout the period of compression. On releasing the pressure upon the aorta normal vascular conditions were again established within a few seconds, but a very rapid decompression generally had the effect of causing a momentary subnormal flow and pressure.

To show that the variations in the carotid bloodflow are fully compensated for, the preceding tests have been amplified by

TABLE II

Carotid bloodflow on compression of abdominal aorta. (Experiment 1, May 5, 1914)

Dog: 14 Kg. Stromuhr in carotid artery

PERIOD	DURATION OF PERIOD	TOTAL QUANTITY OF BLOOD	QUANTITY OF BLOOD	BLOOD PRESSURE (CAROTID ART.)	PROCEDURE
No.	*sec.*	*cc.*	*per sec.*	*mm. Hg*	
17	9.5	19.5	2.05	118.5	None
18	9.1	20.5	2.25		
19	8.2	20.6	2.51		
20	9.0	21.0	2.33		
21	10.1	21.0	2.07		
Average......................			2.24	118.5	
22	6.0	19.8	3.30	140.0	Compression of abdominal aorta
23	4.1	19.2	4.68		
24	4.8	19.4	4.04	145.6	
25	5.2	20.0	3.84		
26	9.8	20.4	2.08	102.8	None
27	10.0	18.8	1.88		
28	9.0	19.9	2.21	115.7	
29	9.1	18.9	2.06		
30	10.2	20.0	1.96		

a series of calibrations of the venous return through the right external jugular vein. In other particulars the experimental conditions are the same as those outlined previously. Experiment I, a part of which is presented in table III, is intended to portray the effects upon the venous influx of increasing the intra-abdominal pressure and experiment II, outlined in table IV, the changes following the digital compression of the abdominal aorta. In examining these data it should be borne in mind that the arterial pressure has been recorded in these experi-

TABLE III

Venous influx in relation to intra-abdominal pressure. (Experiment 1, May 8, 1914)

Dog: 12 Kg. Stromuhr in ext. jug. vein

PERIOD	DURATION OF PERIOD	TOTAL QUANTITY OF BLOOD	QUANTITY OF BLOOD	BLOOD PRESSURE		PROCEDURE
				Ext. jug. v.	Fem. art.	
No.	*sec.*	*cc.*	*per sec.*	*mm.* Hg	*mm.* Hg	
12	12.0	18.9	1.57	0.8	108.4	None
13	12 4	19.6	1.58			
14	11.2	19.6	1.75			
15	9.9	19.7	1.99			
16	12.0	19.4	1.61		107.2	
Average................			1.70	0.8	107.2	
17	8.8	19.8	2.25		118.1	Intra-abdominal pressure raised to 30 mm. Hg
18	9.0	20.0	2.22		124.5	
19	5.7	19.9	3.50	4.5		
20	8.0	19.9	2.48			
21	4.0	18.8	4.70	9.8	114.6	
22	8.0	18.9	2.36			None
23	6.9	19.3	2.79	0.8	107.2	
24	8.9	19.2	2.15			
25	7.8	19.2	2.46			
26	10.2	19.5	1.91			
27	10.4	18.5	1.77			
28	12.0	18.4	1.50	0.8	109.5	
29	14.0	19.0	1.35			
30	13.2	19.2	1.45			
31	12.4	19.2	1.54			
32	9.6	18.9	1.96	3.0	122.5	Intra-abdominal pressure raised to 30 mm. Hg
33	7.4	19.4	2.62		130.0	
34	7.5	20.2	2.69			
35	6.0	20.0	3.33	6.0		
36	5.4	19.5	3.61			
37	6.0	19.6	3.26	8.0	120.0	
38	7.5	18.6	2.48	1.0	109.0	None
39	8.4	18.8	2.23			
40	10.4	19.0	1.82			
41	12.6	19.0	1.51			

ments in the femoral artery and the venous pressure in the right external jugular vein. In order that the reader may be enabled to orient himself more fully, a limited number of the phases of each experiment have been reproduced in the text, the values given in the tables being directly transferable to the curves. In both reproductions the arterial pressure (*F*), as well as the

TABLE IV

Venous influx on compression of abdominal aorta. (Experiment 2, May 15, 1914)

Dog: 13.0 Kg. Stromuhr in ext. jug. vein

PERIOD	DURATION OF PERIOD	TOTAL QUANTITY OF BLOOD	QUANTITY OF BLOOD	BLOOD PRESSURE		PROCEDURE
				Ext. jug. v.	Fem. art.	
No.	*sec.*	*cc.*	*per sec.*	*mm.* Hg	*mm.* Hg	
17	11.0	18.6	1.69	1.0	79.5	None
18	10.2	18.8	1.84			
19	11.4	18.9	1.65			
20	11.0	20.0	1.81			
21	9.6	20.1	2.09		77.2	
Average................			1.86	1.0	77.2	
22	7.6	19.4	2.55	2.0	0.0	Compression of abdominal aorta
23	4.0	19.2	4.80			
24	6.0	19.8	3.30			
25	8.1	19.8	2.44	0.2	69.4	None
26	11.9	20.0	1.68			
27	11.0	19.8	1.80	0.8	78.1	
28	12.0	19.4	1.61			
29	11.4	19.6	1.71			
30	8.0	19.2	2.40	3.5	0.0	Compression of abdominal aorta
31	4.6	18.8	4.08			
32	4.0	18.2	4.55	4.0		
33	5.3	19.8	3.73			
34	8.1	19.0	2.34	0.5	65.0	None
35	12.6	19.8	1.57	1.0	82.0	
36	12.0	19.4	1.61			

venous pressure (*V*), has been recorded above the common abscissa *E*. The time (*T*) is given in seconds. In figure 1 the letters *AB* indicate the period of high intra-abdominal pressure and in figure 2 the time of compression of the abdominal aorta. The record of the stromuhr is marked by the letter *S*.

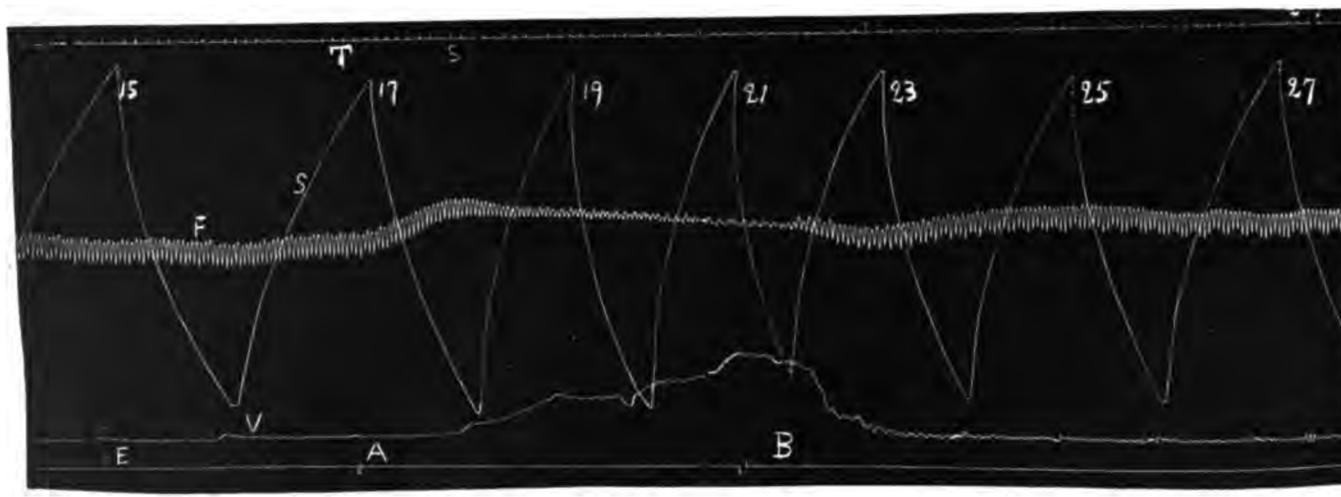

Fig. 1. Intra-abdominal pressure in relation to venous influx. (Curve reduced to 50% its original size.)

These later experiments prove very clearly that the venous bloodstream is subject to the same changes as the arterial, because the inflation of the abdominal cavity, as well as the compression of the aorta, produced increases in the venous flow in no way less pronounced than those encountered on the arterial side. A brief reference to the adjoining curves will prove this point. On examining figure 1, it is found that the bloodflow which previous to the inflation of the abdominal cavity amounted to only 1.70 cc. in a second, increases steadily as the pressure rises, until it reaches its maximal value of 4.70 cc. in a second during phase 21. It is to be noticed that this point coincides with the greatest degree of intra-abdominal pressure attained in this case. The venous pressure shows a rise which is in harmony with the increase in the bloodflow. Its value at the beginning of the experiment was 0.8 mm. Hg and at the end of the inflation 9.8 mm. Hg.

The general pressure, determined in the femoral artery, also presents a rise; eventually, however, its general level declines somewhat below that attained at the beginning of the inflation. This premature decrease implies that the high intra-abdominal pressure serves as an impediment to the flow of the blood to the posterior extremities, but naturally, the degree of pressure here employed (30 mm. Hg) is not sufficient to cause a blocking of the arterial inflow which would permit the femoral pressure to fall below its normal level. In this connection, it must be remembered that the pressure in the carotid system pursues a radically different course. Not being directly exposed to the high intra-abdominal pressure, the carotid arteries remain overfilled throughout the period of inflation and hence, the pressure within these bloodvessels retains its high level rather persistently, an appreciable compensatory fall occurring only if the experiment is continued for a relatively long time.

The phenomena displayed by figure 2 are very similar to those just described. The compression of the abdominal aorta occurring between points *A* and *B*, increased the venous flow from 1.86 cc. in a second to 4.80 cc. in a second. A marked rise in the venous pressure accompanied this change, while the general pressure which was determined in this case in the fem-

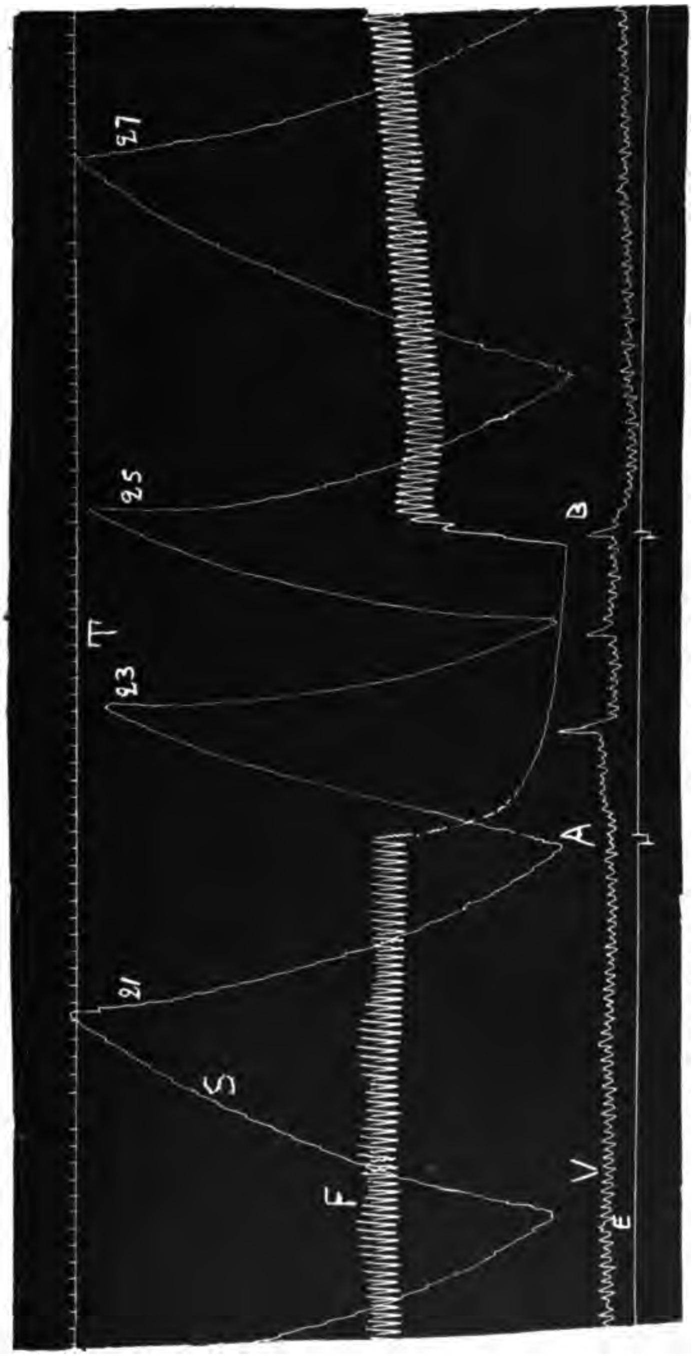

Fig. 2. Compression of abdominal aorta on venous influx. (Curve reduced to 80% its original size.)

oral artery, presented at this time a pronounced fall for obvious reasons. This curve also shows a compensatory fall in the pressures and the flow subsequent to point *B*, which was caused, as has been stated previously, by releasing the compression too suddenly.

The experiments briefly referred to at the beginning of this paper have shown that the downward movement of the diaphragm occasioned by the stimulation of the phrenic nerves, possesses a rather perplexing influence upon the distribution of the blood, in that it lessens the venous return from the posterior extremities and increases the flow from the external jugular veins. Clearly, this phenomenon cannot be explained satisfactorily upon the basis of an inspiratory fall in the intra-thoracic pressure, because the aspiratory force does not act in the same way upon the two venae cavae. A much more plausible reason for it is to be found in the increased intra-abdominal tension coincident with the descent of the diaphragm; which hinders the flow of the blood through the posterior channels of the body and greatly favors the circulation through bloodvessels of the head.

The question of whether an appreciable transfer of blood also takes place when the respiratory motions are shallow and of brief duration, cannot be answered with certainty. The present experiments suggest, however, that this mechanism is brought into play as soon as the movements become deep and prolonged or whenever an undue resistance is encountered by the descending diaphragm.

While clearly recognizing the important bearing of the intra-abdominal pressure upon the distribution of the blood, our attention must also be directed to the influence of the suction action of the inspiratory motions upon the venous return and especially upon that from the fore part of the body. Henderson and Barringer[6] seem to attach only a slight importance to this factor, because they state that "the suction induced in the intra-thoracic veins by the negative pressure of the thorax has by some writers been supposed to draw the blood onward from the extra-thoracic vessels. If so, the inspiratory increase of the

[6] Henderson and Barringer: American Journal of Physiology, xxxi, 1912, 402.

negative pressure would augment the venous supply to the right heart. It seems now to be generally recognized, however, that a suction cannot be transmitted to any considerable distance through the veins. They are too readily collapsible; the pressure within them is too low and the flow is too slow." It seems to me that these conclusions are based solely upon evidence which, at best, could be true only in a relative way and hence, it must be regarded as doubtful whether the factors here cited are sufficiently powerful to prevent an influence of this kind from being brought to bear upon the venous current.

I may be permitted to refer in this connection to certain experiments of my own[7] which, although not mentioned by the authors just cited, possess a direct bearing upon this question. These tests have shown that the respiratory motions induce inspiratory augmentations and expiratory retardations in the venous flow in the external jugular vein which preserve a direct relationship to the depth of the movements, and, that similar variations accompany the contractions of the diaphragm when induced by moderate stimulations of the phrenic nerves. Thus, having proved that the influences dependent upon changes in intra-thoracic pressure, must indeed be reckoned with, the suppositions of Henderson and Barringer cannot be considered as valid.

As these respiratory variations occur at intervals without that the venous flow suffers a marked retardation and, moreover, as the flow continues unabated when the respiratory movements are made to cease during brief periods of time, I felt justified in concluding further that the heart is by far the most important factor while respiration plays only a secondary part. The latter merely assists in propelling the blood into the more central venous channels. Henderson and Barringer[8] state that "according to our view the utmost assistance that respiration can afford to the circulation is to maintain a venous pressure sufficient to distend the right ventricle as rapidly as it closes and as fully as the duration of diastole allows." I confess that, in consideration of the experimental data submitted by me

[7] Burton-Opitz: Am. Journ. of Physiol., vii, 1902, 435.
[8] Loc cit., p. 400.

twelve years ago, I cannot do otherwise than fully coincide with this statement.[9]

The importance of the respiratory movements increases with every increase in the frequency and depth of the motions and hence, a state of activity may be reached at times during which the respiratory effects become indeed of surpassing importance to the venous flow and pressure. The evidence supplied by the present experiments therefore seems to favor the view that the chest and abdomen may act as a force and suction pump.

The question of whether a greater quantity of blood is furnished to the heart during inspiration, cannot be answered with certainty, because it must be considered as possible that the augmented flow from the fore part of the body is counterbalanced by a decrease in the influx from the inferior vena cava. Eppinger and Hofbauer[10] have pointed out that the lumen of this bloodvessel is lessened during inspiration. Under ordinary conditions this change would serve as an impediment to the venous return. While proving this supposition regarding the flow to be correct, the preceding tests have also shown that the period of lessened influx during the descent of the diaphragm is ushered in by a brief augmentation which may partially compensate for the decrease occurring later on.

For this reason I am rather inclined to believe that, on the whole, the cardiac vestibule and central venous channels receive a greater supply of blood during the inspiratory movement. Providing that the heart is sufficiently receptive at this time, it may be conjectured that the degree of venous pressure existing at the end of inspiration greatly favors an augmented influx into the auricle. But again, it is conceivable that the inspiratory and expiratory variations in the flow are counterbalanced and that the flow through the auricular orifice is therefore constant. I mention this possibility merely to show that a constancy of flow, such as Henderson and Barringer, as well as Piper,[11] assume to be present, could also be obtained without taking the "effective pressure" into consideration.

[9] See Burton-Opitz, This Journal, vii, 1902, 446.
[10] Eppinger and Hofbauer: Zeitschr. f. klin. Med., lxxii, 1911, 154.
[11] Piper: Archiv f. Anat. u. Physiol., 1913, 396.

2. Each article should conclude with a brief summary of results, suited to the needs of reference journals.

3. Reference to literature cited should contain, first, the name of the author or authors; second, the name of the journal or book; third, the year; fourth, the volume number and fifth, the page. Thus, Meltzer: American Journal of Physiology, 1908, xxi, 449. References should be numbered serially and be gathered at the end of the paper.

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CONTENTS

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1. While the length of articles cannot be specified, they should not run to excessive length especially by inclusion of unessential tables and protocols which add materially to the cost of publication.

Continued on page 3 of cover

THE AMERICAN
JOURNAL OF PHYSIOLOGY

VOL. 36 JANUARY 1, 1915 No. 2

STUDIES ON CEREBRO-SPINAL FLUID

VIII. The Effect of Pituitary Extract Upon Its Secretion (Choroidorrhoea)

LEWIS H. WEED and HARVEY CUSHING

From the Laboratory of Surgical Research, Harvard Medical School, Boston

Received for publication October 5, 1914

I. INTRODUCTORY

In the course of these studies we first devoted ourselves to the determination of the various pathways of escape for the fluid from the subarachnoid spaces, and it was not until some comprehension of these processes was afforded[1] that our attention was turned to the question of its elaboration. In a preceding paper it was pointed out that the fluid need not be considered as solely a product of the choroid plexuses, for evidence was presented that accretions to the fluid may be derived from the important perivascular system, which, in accordance with our conception, carries the fluid waste products of nerve cell activity toward the subarachnoid spaces.[2] The question naturally arose, do the drugs which appear to increase the flow of cerebro-spinal fluid act directly on the choroid plexuses or is their influence exerted on the cerebral capillaries in such a way that an increased amount of fluid is poured out through the perivascular lymphatics? In the attempt to answer this question it was necessary to devise some method by which the secretion from the choroid plexuses themselves could be secured apart from that possibly derived from other sources. This was at first

[1] Studies on cerebro-spinal fluid. Nos. I–VII. Jour. Med. Research, 1914. xxxi, 1 to 176.

[2] Ibid, No. IV. The dual source of cerebro-spinal fluid. Jour. Med. Research, 1914, xxxi, 109.

accomplished by the method, described in an earlier paper, of catheterizing the third ventricle through the aqueduct of ylvius after opening the cisterna magna and exposing the fourth ventricle; and though information of value was thus secured the method is open to the many objections which apply to all extensive suboccipital procedures—the severity of the operation with its considerable trauma and loss of blood, the unavoidable immediate escape of a considerable amount of pre-formed fluid, and, in case an attempt is made to tap the cistern without the preliminary operative exposure of the occipito-atlantoid ligament, the liability of pricking the brain stem, with more or less resultant subarachnoid bleeding.

Some studies by Wegefarth[3] had demonstrated the absence of complications following a mid-line puncture of the third ventricle directly through the longitudinal sinus, and consequently we came to employ this device in our later observations (e.g. protocol of experiment LIV), for under these circumstances the operative steps preliminary to securing a uniform record of drops are greatly simplified, and as the cranial chamber remains intact there is no immediate escape of a large amount of fluid.

We have ventured to introduce the term *choroidorrhoea* as a convenient designation of a condition of increased secretory activity of the plexuses.

II. REVIEW OF LITERATURE

The early suggestion by Faivre[4] and by Luschka[5] some sixty years ago, that the choroid plexuses give rise to the fluid, was based entirely upon the presumably glandular character of these vascular structures. The first definite proof of the correctness of their assumption was not afforded until Capelletti[6] in 1900

[3] Ibid, No. VI, The Establishment of Drainage of Intra-ocular and Intracranial Fluids into the Venous System. Jour. Med. Research, 1914, xxxi, 149.

[4] Faivre, E.: Des granulationes meningiennes. Paris Thesis, 1853.

[5] Luschka, H.: Die Adergeflechte des menschlichen Gehirns, Berlin, 1855.

[6] Capelletti, L.: L'efflusso del liquido cerebro-spinale dalla fistola cefalorachidiana in condizioni normali e sotto l'influenza di alcuni farmaci. Atti dell' Accad. d. Scienze Med. e Nat. d. Ferrara, 1900, lxxiv, 85.

introduced pharmacological agents into the study of cerebro-spinal fluid production. He demonstrated a marked increase in the rate of flow of the fluid under the influence of muscarine, pilocarpine and ether, and a slowing under the influence of atropine. Two years later Pettit and Girard,[7] as reported in their exceptional monograph, correlated the conditions of increased production of the fluid with the occurrence of definite histological changes in the cells of the choroid plexuses, thus affording more convincing proof than had been presented that these structures are concerned with the elaboration of the fluid. Subsequent studies by Meek,[8] Findlay,[9] Yoshimura[10] and a number of others served to place the secretory function of the plexuses upon a still more solid basis, and Mott,[11] goes so far as to term these structures the "choroidal glands."

More recently Dixon and Halliburton[12] have presented reports dealing with the rate of production of cerebro-spinal fluid under the influence of various pharmacological agencies unemployed by Capelletti. They were apparently the first and only investigators to report upon the effects of pituitary extracts, which in their hands gave entirely negative results. They made, however, the significant observation that a marked increase in the secretion follows the intravenous injection of dried extracts of the choroid plexuses and that a slight response also occurs to extracts of brain substance itself. This demonstration of an apparent hormone action upon the production of the fluid will doubtless incite others to work in this field, and many other

[7] Pettit, A., and Girard, J.: Sur la fonction sécrétoire et la morphologie des Plexus Choroid des ventricules latéraux du Systeme nerveux central. Paris, 1902.

[8] Meek, W. J.: A study of the choroid plexus. Jour. Comp. Neurol. and Psych., 1907, xvii, 286.

[9] Findlay, J. W.. The choroid plexuses of the lateral ventricles of the brain: their histology normal and pathological. Brain, 1899, xxii, 161.

[10] Yoshimura, K.: Die histochemische Verhalten des menschlichen Plexus choroideus. Arbeiten a. d. neurol. Inst. a. d. Wien Univ., 1909, xviii, 1.

[11] Mott, F. W.: "The Oliver-Sharpey lectures on cerebro-spinal fluid." Lancet, 1910, ii, 1.

[12] Dixon, W. E., and Halliburton, W. D.: The action of the choroid plexuses on the secretion of cerebro-spinal fluid. Jour. Physiol., 1910, xl, 30; Cerebro-spinal fluid, I. The secretion of the fluid. Ibid, 1913, lxvii, 215.

tissue extracts will undoubtedly be tested.[13] Most of our own experiments were made before the publication of Dixon and Halliburton's complete paper, and our experiences with ether, adrenaline, pilocarpine and other substances are more or less in accord with those of our predecessors. In this report we shall dwell exclusively upon the effects observed with pituitary extracts.

III. METHODS EMPLOYED

The rate of production of cerebro-spinal fluid has been studied by previous observers in various ways. Cavazzani[14] established a cerebro-spinal fistula for the purpose, but in the majority of cases hollow needles or cannulae have been introduced into the great subarachnoid cistern beneath the occipito-atlantoid ligament or elsewhere into the subarachnoid spaces of the spinal canal.

The results obtained by these customary methods, as has been indicated, are susceptible to misinterpretations, and in the attempt to avoid them, in our early studies use was made of ventricular catheterization through the iter. The small paraffined silk catheters employed were calibrated in terms of millimeters of water (i.e. the height of a column of water sufficient to cause fluid to drop from the end); and after operative exposure of the fourth ventricle practise makes it possible to safely introduce one of them along its floor and through the aqueduct of Sylvius into the third ventricle. The resistance of the catheters was such that pressures approximating the normal were maintained within the ventricles. In this way the normal intraventricular pressure conditions were supposedly not interfered with, and the results obtained promised a greater degree of reliability than when, under the usual conditions of such an experiment, the intraventricular tension is allowed to fall almost to zero.

[13] Frazier reports briefly upon experiences in this direction and states that thyroid preparations appear to inhibit the secretion. (The cerebro-spinal fluid as a problem in intracranial surgery. Jour. Amer. Med. Assn., 1914, lxiii, 287.)

[14] Cavazzani, E.: La fistola cefalorachidiana. Atti dell' Accad. d. Scienze Med. e Nat. di Ferrara, 1899, lxxiii, 27.

The chief disadvantage of this method lay in the severity of the preliminary operative procedure, and indeed the mere free exposure of the occipito-atlantoid ligament in the canine with the subsequent introduction of a needle into the posterior cistern, after the manner usually employed, is open to the same objection. To avoid the loss of blood and shock incidental to this extensive exposure Dixon and Halliburton inserted the needle through the soft parts and ligament into the cistern after making little more than a skin incision; but there is always danger under these circumstances of pricking the nervous axis and of causing subarachnoid bleeding. Even with a successful puncture of the cistern by this method and the use of a calibrated needle we felt, for the reasons given, that our observations would be more dependable could our records be made from the intraventricular rather than from the subarachnoid fluid spaces.

We finally hit upon the far simpler method of introducing the calibrated needle into the third ventricle by a callosal puncture—a method which, besides its simplicity, has the additional advantage of tapping the ventricle, with the skull and meninges left intact, so that the intracranial physical conditions remain unaltered and there is no primary gush of fluid such as occurs when the cistern is entered through the occipito-atlantoid ligament.

In this procedure a mid-line perforation through the bone and down to the longitudinal sinus is made with a small drill the size of the calibrated trocar which is to follow it, so that the latter when introduced stays securely in place. This small opening is occluded with wax or plastacene and the trocar and cannula are then introduced directly through the sinus and alongside the falx into the third ventricle—a depth of 2.5 or 3.5 cm., depending on the size of the animal (fig. 1). The trocar is then withdrawn from the cannula and clear fluid, without the usual primary outrush, begins to drop at regular intervals which can be recorded.

In all such observations the anaesthetic requires particular attention. As both chloroform and ether are known to affect the flow of cerebro-spinal fluid, they must be administered more

evenly than is possible when either of these volatile anaesthetics is given in a cone or by the open drop method. Only by the employment of intratracheal insufflation with an unchanging tension of the anaesthetic can a long enduring, constant level of blood pressure be insured during the number of hours necessary for the experiment.[15]

The experiments were conducted with the subject (dog or cat) in the ventral position. The arterial blood pressure as a rule was recorded from the carotid, for though the femoral was

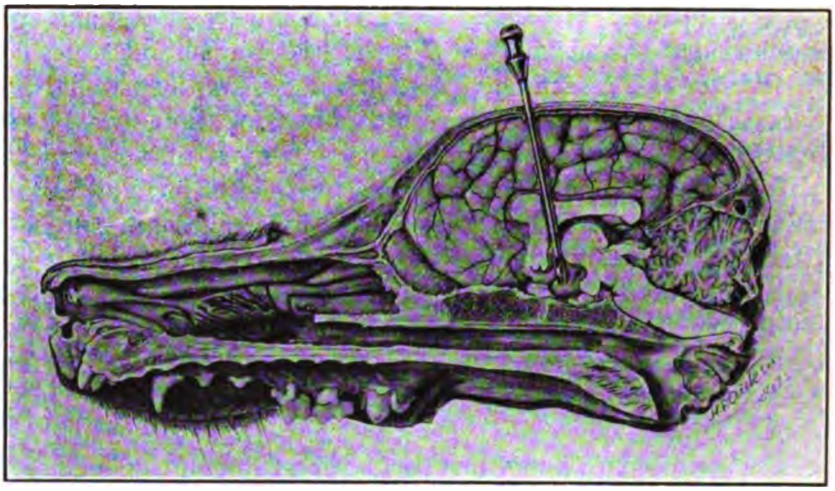


Fig. 1. Sagittal Section of Canine Skull, Showing Point of Election for Tapping Intraventricular Fluid.

occasionally used, the ligation of one carotid apparently has no effect on the secretion of the fluid. The respiration tambour recorded inspiration by an upward stroke. In certain observations the urinary outflow was also included on the record, the drops of urine, issuing from a catheter inserted in a ureter extraperitoneally in the loin, being indicated by an appropriate signal.

[15] Dixon and Halliburton attempted to overcome these difficulties by using morphine and urethane as an anaesthetic. We have had less success with this method.

All injections were made intravenously, usually into the small saphenous vein, readily exposed on the posterior aspect of the dog's leg and by far the most satisfactory vessel in which to make repeated injections with an animal in this position.

IV. THE EFFECT OF PITUITARY EXTRACTS UPON THE FLUID PRODUCTION

It is well known that intravenous injections of suitable amounts of posterior lobe preparations cause a rather prolonged rise in arterial pressure, usually preceded by an initial fall of variable duration. This typical haemodynamic response, first noted by Schäfer,[16] was attributed by Howell[17] to substances confined to the pars nervosa. Repeated injections of these extracts at short intervals tend in the main to increase the depressor phase at the expense of the pressor.

Experience has taught us that there is great variability in these haemodynamic responses to posterior lobe extracts, whether due to the method of preparation, to the state (possibly seasonal) of inactivity or otherwise of the gland from which the extracts are prepared, or to the individual susceptibility to the extracts (possibly again seasonal or periodic) on the part of the animals on which they are tested. Undoubtedly many of the contradictory results of the pharmacological studies on hypophysial extracts are attributable to these several sources of confusion. Moreover there are in all likelihood several active principles or hormones, rather than a single one, contained in the gland, and, by the particular method of preparation employed, one or another of the substances may fail to be extracted even should it actually have been present in significant amounts in the fresh glands. Thus one may secure preparations in which either the presser or the depressor response is paramount, or preparations in which there is no haemodynamic response whatsoever, and yet the same preparations may cause marked diuresis, or glycosuria,

[16] Schäfer, E. A.: The physiological effects of extracts ot the pituitary body. Jour. Physiol., 1899–1900, xxv, 87.

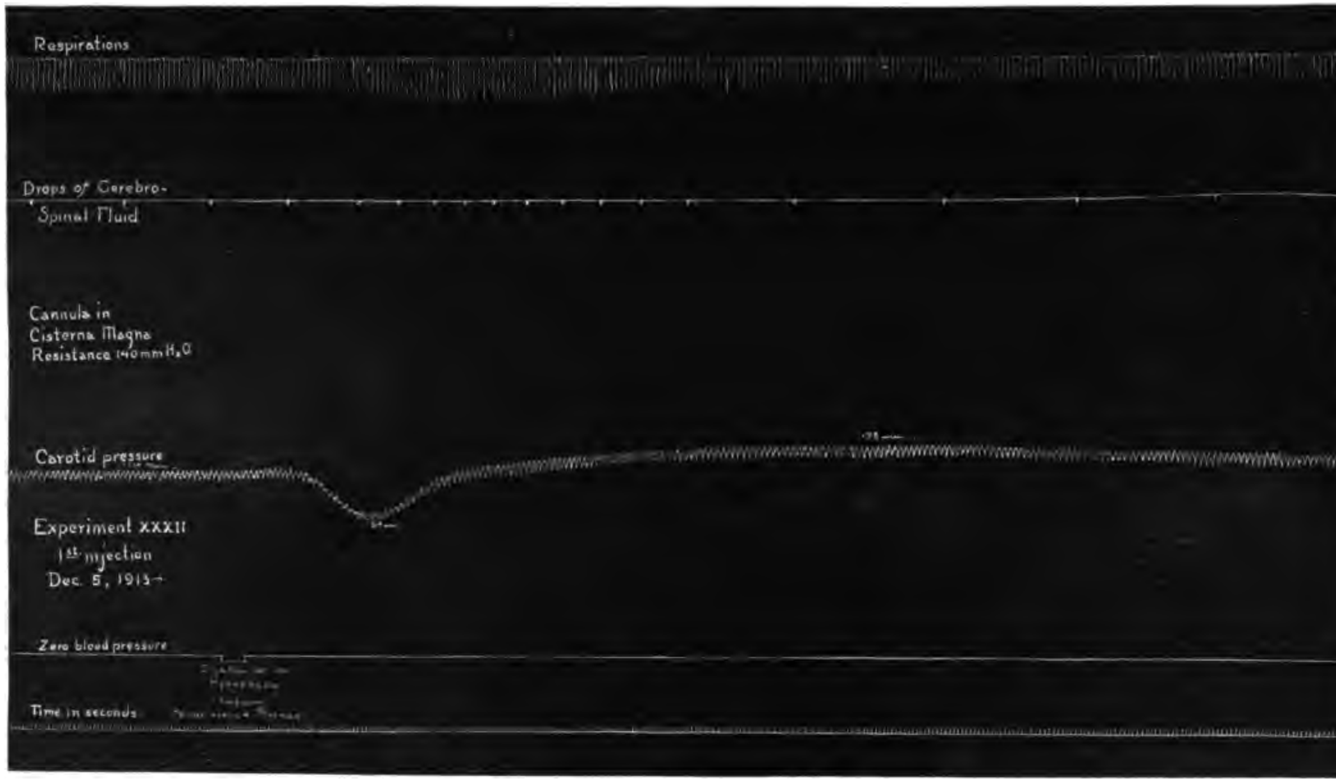
[17] Howell, W. H.: The physiological effects of extracts of the hypophysis cerebri and infundibular body. Jour. Exper. Med., 1898, iii, 245.

or powerfully contract smooth muscle, and so on. We have observed, too, that successive proprietary samples extracted presumably by the same technical method are most variable in their potency, so that until the pharmacologist can actually isolate, and perhaps synthesize, the substance or substances in question, making it possible to estimate accurately the dosage, negative reactions with the substances at hand are far less significant than positive ones.

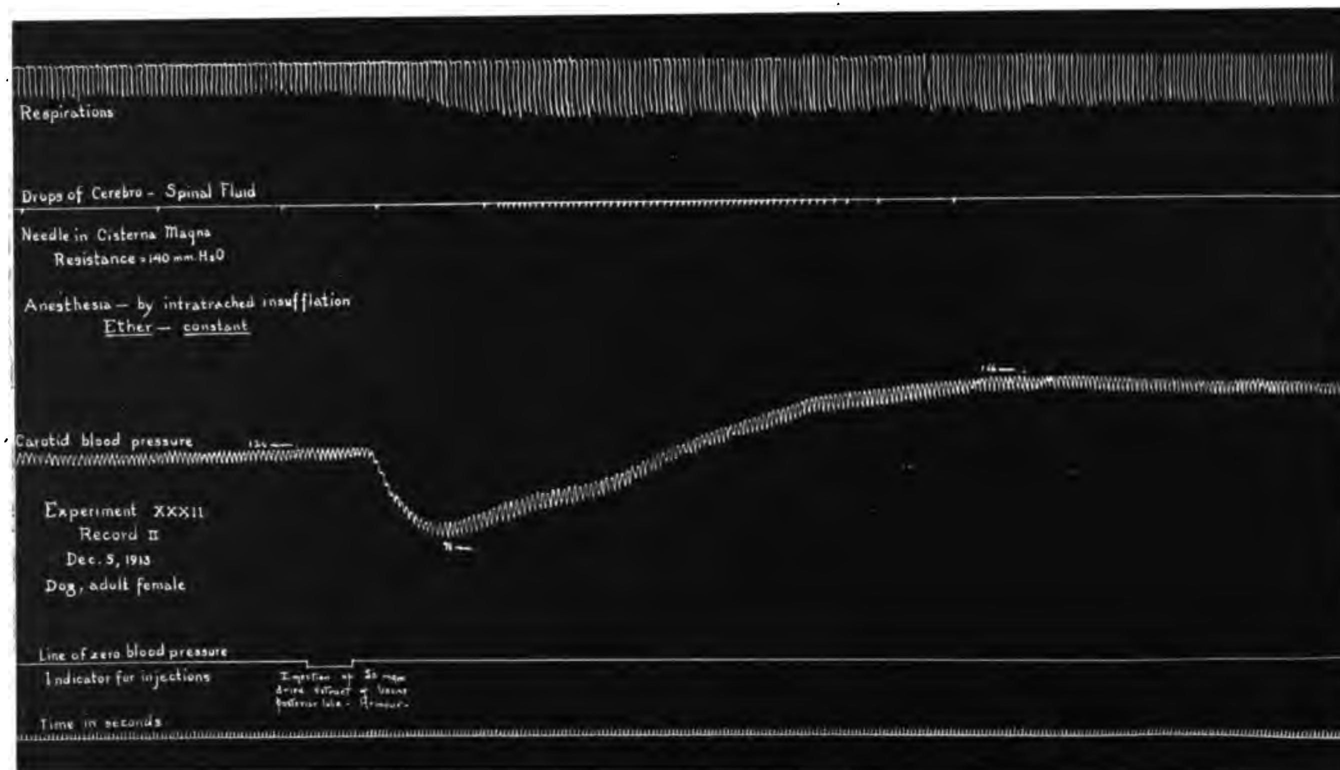
As it is true of the haemodynamic or diuretic responses, so in the case of the cerebro-spinal fluid production great variation has been observed with the different extracts which have been used. The most active preparations in our experience have been the dried extracts of fresh bovine posterior lobes prepared in this laboratory and also some powdered extracts dried *in vacuo* for us by Armour and Company. We have compared these dried extracts with the various fluid preparations of the different pharmaceutical firms (Parke, Davis and Company's "Pituitrin;" Lucius, Meister, and Brünig's "Hypophysin;" Armour and Company's "Pituitary Liquid," and the "Pituitary Extract" of Schering and Glatz), and though none of them has been as potent as the dried extracts, nevertheless an augmentation of the flow of cerebro-spinal fluid has been obtained at one time or another from each of these fluid preparations.

A fairly typical though slight response to the injection of 1 cc. of "Hypophysin" is shown in Record I, the fluid in this experiment (No. XXXII) issuing from a calibrated needle in the cisterna magna with a regular outflow established at the rate of a drop about every twenty seconds. Following the injections the characteristic though slight vasomotor response (with a primary depressor phase from 114 mm. to 84 mm. and a subsequent rise to 128 mm. Hg.) occurred, with a moderate acceleration of fluid outflow at the rate of a drop every eight seconds for the succeeding two minutes, followed by a subsequent slowing of the pre-injection rate.

Subsequently (cf. Record II) in the course of this same experiment (No. XXXII), after the re-establishment of a regular fluid outflow, at the rate of a drop every thirty seconds, an injection



Record I. **Experiment XXXII. December 5, 1913. Dog. Intratracheal anaesthesia: cannula in cisterna magna: slight increase in flow after injection of 1 cc. "Hypophysin," with slight blood pressure response.**



Record II. Conditions of experiment as in Record I: marked temporary choroidorrhoea with more pronounced vasomotor response, following the injection of 50 mgm. of dried bovine posterior lobe extract.

of 50 mgm. of the dried bovine extract in 3 cc. of salt solution gave a more pronounced response, with the typical fall (from 126 to 78 mm. Hg.) and subsequent rise (to 166 mm.) in blood pressure, the former phase being somewhat more prolonged than usual. As will be seen, the onset of the sudden outpouring of cerebro-spinal fluid corresponded with the period of lowest blood pressure and ceased at the crest of the pressor wave a minute and a half later, following the escape of 60 drops of fluid, after which there was an abrupt cessation in the flow. Coincident with this period of supposed choroidorrhoea a slight amplitude of the respiratory stroke is registered,[18] and without further data one might be inclined to attribute the response to the physical expulsion of pre-formed fluid brought about by an increase of intracranial tension from asphyxia or some other cause. On this basis, however, one would not have expected the fluid to be expelled during the depressor, but rather through the pressor, phase, as is the case after compression of the jugulars, when there is apt to be a temporary flow from the cannula, due undoubtedly to mechanical rather than secretory influences.

It will be seen from the succeeding two records, taken from other animals in which the characteristic initial depressor phase in the haemodynamic responses did not occur, that very similar responses on the part of the cerebro-spinal fluid were nevertheless elicited.

In Record III, from a canine experiment (No. LI) the fluid, at the time of the injection of 100 mgm. of dried bovine extract, was issuing from the cistern catheter at the rate of 8 drops a minute, and immediately upon the injection the rate quickened to 50 drops for the following minute, this response being inaugurated before the initial stage of the haemodynamic pressor

[18] Dixon and Halliburton (*loc. cit.*) state that an increase in the rate or amplitude of respiration facilitates the normal processes of escape of the fluid with consequent decrease in the flow from the cannula. Here the marked increase in fluid outflow is synchronous with the increase in the respiratory movements. These suction effects of respiration do not appear to us to have influenced the responses in any of the experiments, in most of which the normal pathways of absorption, via the meninges, were excluded by the methods employed, of intraventricular insertion of the catheter or cannula.

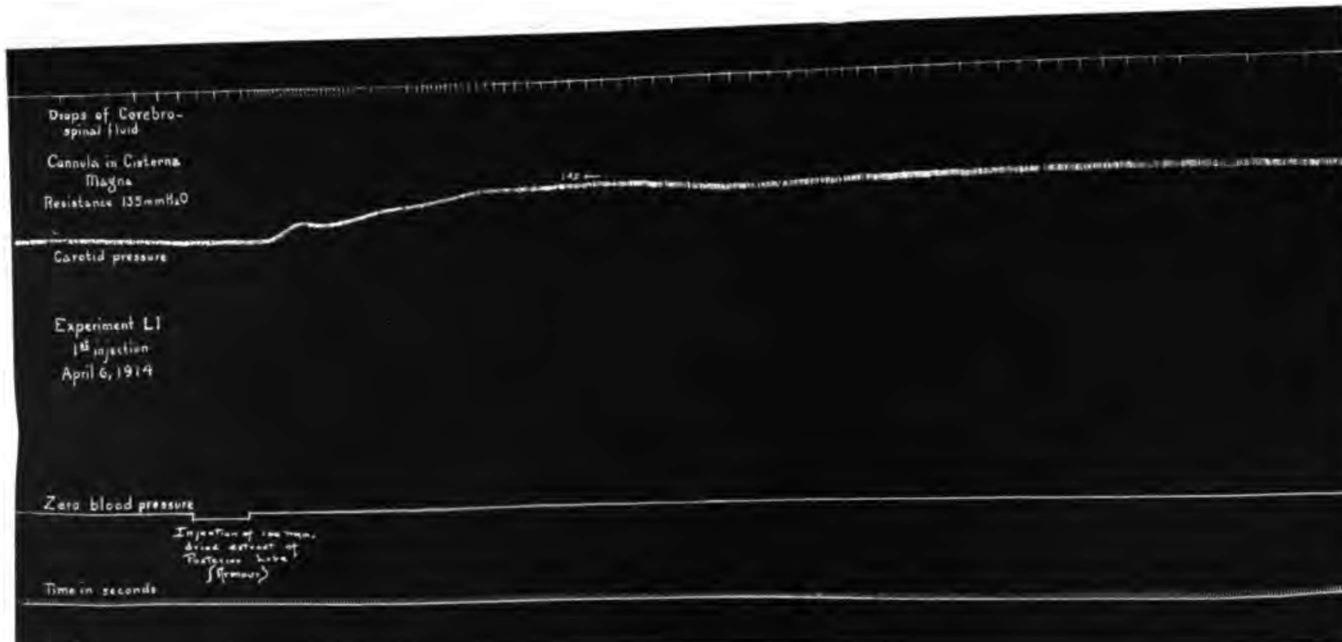
change set in. There was a subsequent gradual retardation of the flow, with temporary complete cessation after seven minutes. The respirations were not recorded in this experiment but here again, as in Record II, the period of increased flow coincided more or less sharply with the period of rising blood pressure. The following record, however, shows that the choroidorrhoea may occur with a persisting level of blood pressure.

In Record IV, from a dog with a calibrated catheter in the aqueduct, (Experiment No. XXV) is shown an extreme temporary choroidorrhoea. The animal had already received one injection of an extract which after a slight response had been followed by a period of cessation in the flow. On this second occasion 50 mgm. of dried bovine posterior lobe extract was given, causing an enduring primary pressor response (from 116 to 158 mm. Hg.) with an immediate outpouring in the course of the next 4 minutes of 128 drops (about 8 cc.) of fluid—far more than the canine ventricles normally contain. This was followed in turn by a second prompt cessation without any apparent change, as will be noted, either in blood pressure or respiration.

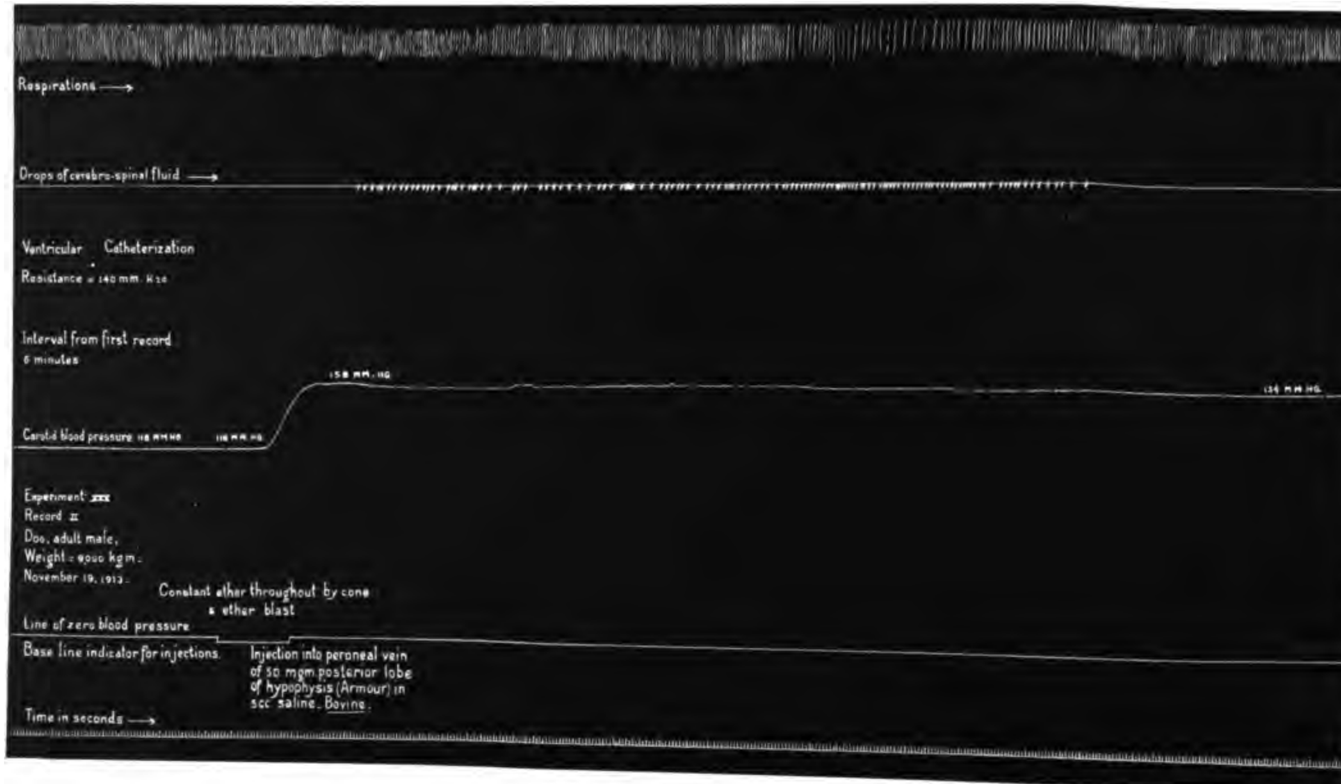
In order to emphasize still further the lack of dependence of these fluid responses upon haemodynamic or other obvious mechanical changes in intracranial tension the following protocol may be given in full.

Experiment LIV. July 17, 1914. Adult female 10.5 kilo dog. *9.15 a.m.* Intratracheal anaesthesia: insertion of catheter in right ureter, exposed extraperitoneally by posterior incision in flank: introduction of blood pressure cannula in right carotid: exposure of posterior saphenous vein in left leg: ventriculo-callosal puncture: attachment of carotid cannula to manometer and kymographion. Blood pressure 136 mm. Hg., practically uninfluenced by respiration, which is panting, about 80 to the minute (and which continued so during the experiment). Cerebro-spinal fluid gradually slowing from initial rate of 22 drops per minute to about 10 per minute at time of first injection. Urine flowing at rate of 3 drops per minute from the single ureter: no sugar in first specimen.

10.17 a.m. First injection (cf. *Record V*): 4 cc. of a concentrated 20 to 1 solution of bovine cerebro-spinal fluid, followed by a marked



Record III. Experiment LI. April 6, 1914. Dog. Intratracheal anaesthesia: calibrated catheter in cisterna magna: acceleration of flow with pressor response and no initial depressor phase.



Record IV. Experiment XXV. November 19, 1913. Dog. Catheterization through aqueduct: marked temporary choroidorrhoea associated with long-enduring and stationary pressor response.

depressor response (from 136 to 64 mm. Hg.) and slight subsequent and prolonged rise (to 150 mm. Hg.) without apparent influence on the rate of cerebro-spinal fluid flow. An immediate temporary check, however, occurred in the outflow of urine.

10.25 a.m. *Second injection* (cf. *Record VI*): 100 mgm. of posterior lobe powder (Armour) dried *in vacuo* at 38°. Prompt cerebro-spinal fluid response lasting 3 minutes, with increase in drops from 4 per minute to 13 per minute (over 2 cc. in all), coincident with an extreme depressor response. This was followed by an abrupt and prolonged rise in blood pressure (to 176 and finally 192) with a cessation in cerebro-spinal flow, which endured for about thirty minutes, when (at *10.49 a.m.*) there was a slow return of drops, at the rate of one per minute.

Seven minutes after the injection (i.e. at *10.32 a.m.*) an abrupt polyuria started up and continued for the following half hour, during which time there was such a rapid flow that 27 cc. were collected from the single ureter, and probably double that amount was secreted from the two kidneys. The urine contained a high percentage of sugar.

11.12 a.m. After a long-enduring pressor response the carotid registration is again at 150. Polyuria continues. A cerebro-spinal fluid drop every 2 minutes. *Third injection:* 4 cc. of "Hypophysin," causing a slight vasomotor response (150 to 116 to 172 mm. Hg.) and coincident cessation of cerebro-spinal fluid for the following 13 minutes, after which time (at 11.25) fluid again began to appear, and continued at the rate of about 3 drops per minute for the interval between 11.30 a.m. and 12.40 p.m., during which time the drum was stopped and a new record prepared.

12.50 p.m. Carotid pressure at constant level of 148 mm. Cerebro-spinal fluid drops regular, 3 per minute. *Fourth injection:* 100 mgm. of dried posterior lobe extract (laboratory preparation) followed by marked vasomotor response with a double depressor phase (148 to 120: 158 to 126 mm.) and subsequent prolonged rise (at 162 mm.): also by a temporary acceleration of cerebro-spinal fluid outflow and subsequent slowing to about one drop per minute; also by a temporary cessation of urine for 4 minutes and then a sharp polyuria at the rate of 9 drops per minute.

1.05 p.m. *Fifth injection:* 5 cc. of the Schering preparation followed by a very slight vascular response (132 to 120 to 138 mm.) with a slight acceleration of the cerebro-spinal outflow but no effect on the steady flow of urine.

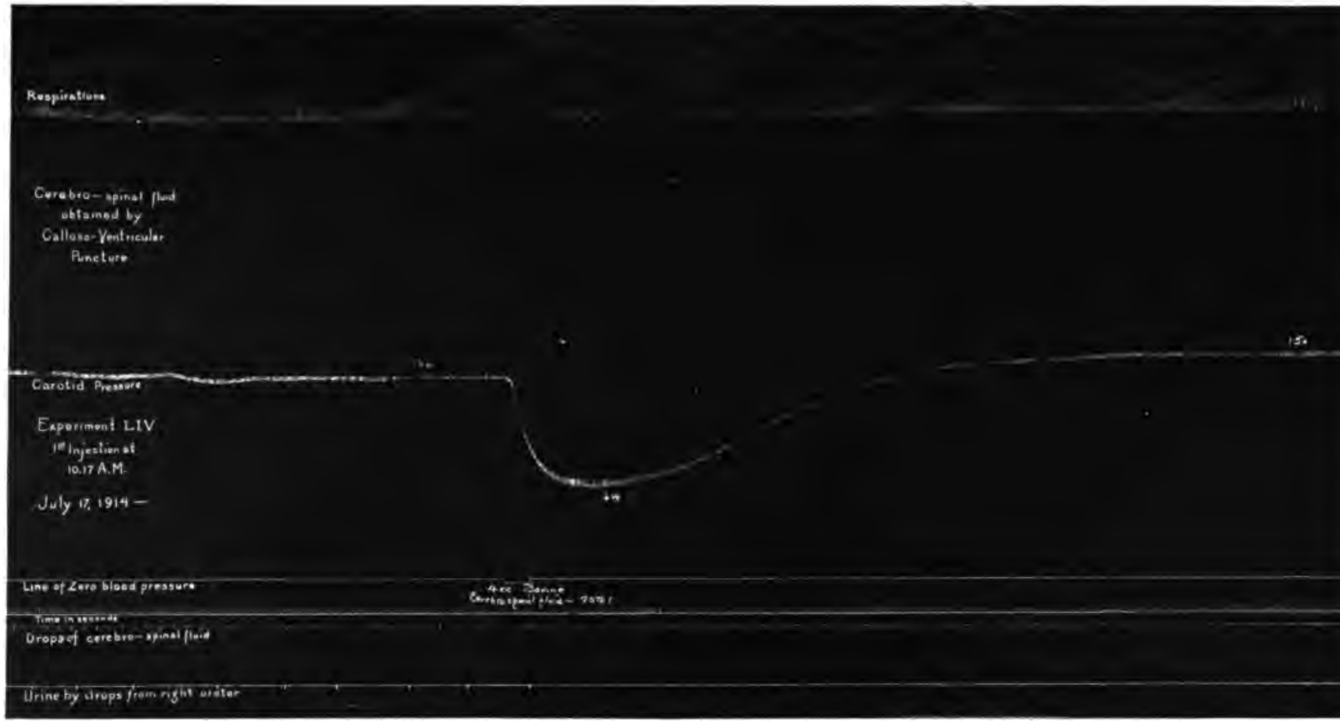
1.17 p.m. Sixth injection. Repetition of 100 mgm. Armour preparation dried *in vacuo*, followed by an immediate quickening of cerebro-spinal fluid drops from 1 to 6 per minute and a temporary 3 minute cessation of urine in association with a marked vasomotor response, (with primary fall from 142 mm. to 114 mm. and subsequent prolonged rise, finally reaching 196 mm. Hg.).

1.30 p.m. Seventh injection. Repetition of the above with a similar good response associated with definite choroidorrhoea (7 drops per minute), followed by practical cessation.

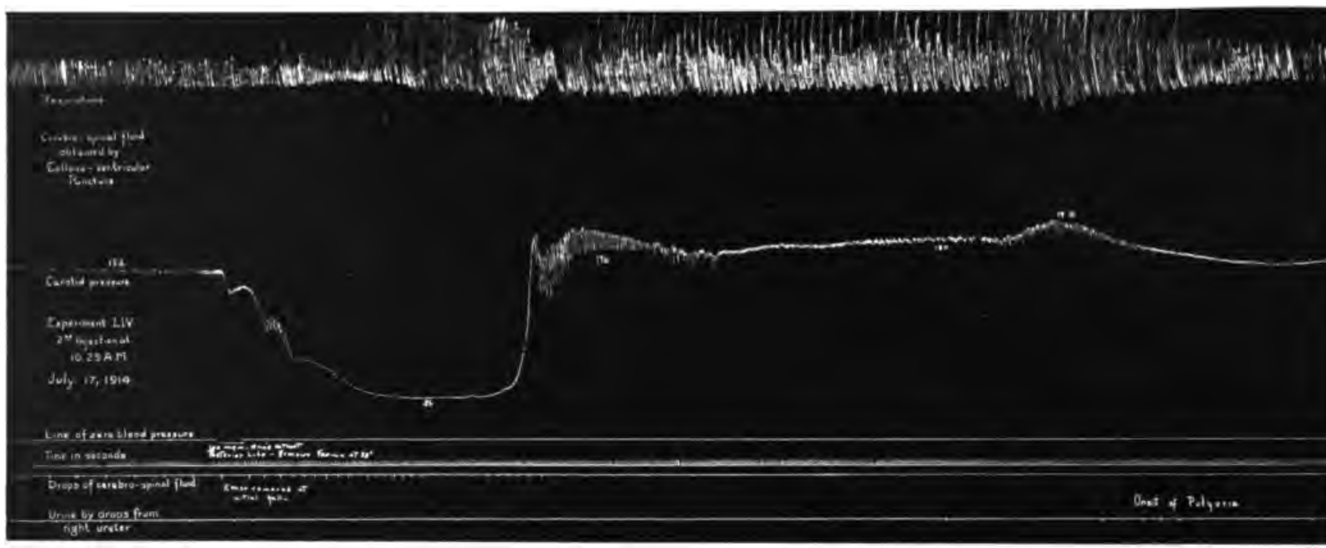
1.35 p.m. Eighth injection: 3 cc. of ether followed by a sharp fall in blood pressure from 156 to 64 mm. Hg., accompanied by the appearance of a few drops of cerebro-spinal fluid.

1.40 p.m. Ninth injection: 5 cc. of ether to produce fatality, followed by a prompt fall of blood pressure to zero, and respiratory cessation, associated with an immediate choroidorrhoea (14 drops in the first minute) which continued for twenty-one minutes after death: 4.1 cc. of cerebro-spinal fluid secured in all. The urine, which up to this time had been dropping rapidly, ceased abruptly, coincident with the fall of the blood pressure to zero.

This protocol emphasizes a number of things: the variability of the responses in the same animal to different extracts: the fact that it is the substance injected which causes the increased fluid outflow rather than the variations in blood pressure associated with possible changes in the physical conditions within the skull (cf. Records V and VI): the fact that even in a prolonged experiment (in this case nearly four hours) the secretory activity need not be exhausted by repeated injections, provided that an extreme choroidorrhoea has not been provoked at any one time: and further that even when the responses to pituitary extracts have weakened, an injection of ether may arouse an active choroidorrhoea. That the pituitary substances, particularly the dried extracts, were active in other directions than on the cardio-vascular system is shown by the glycosuria following the first injection, as well as by the diuretic responses, and it may be noted that the latter effect after each potent injection was usually preceded by a latent period of two or three minutes, whereas the choroid effect was immediate and often briefly anticipated the characteristic haemodynamic response.



Record V. **Experiment LIV. July 17, 1914. Dog. Intratracheal anaesthesia: callosal puncture. Showing marked depressor response (to first injection of 4 cc. bovine cerebro-spinal fluid in 20 to 1 concentration), without effect on cerebro-spinal outflow.**



Record VI. Experiment LIV (second injection). Showing immediate temporary choroidorrhoea associated with depressor response after injection of 100 mgm. posterior lobe extract (Armour) dried *in vacuo*. Polyuria (with glycosuria) after latent period of seven minutes.

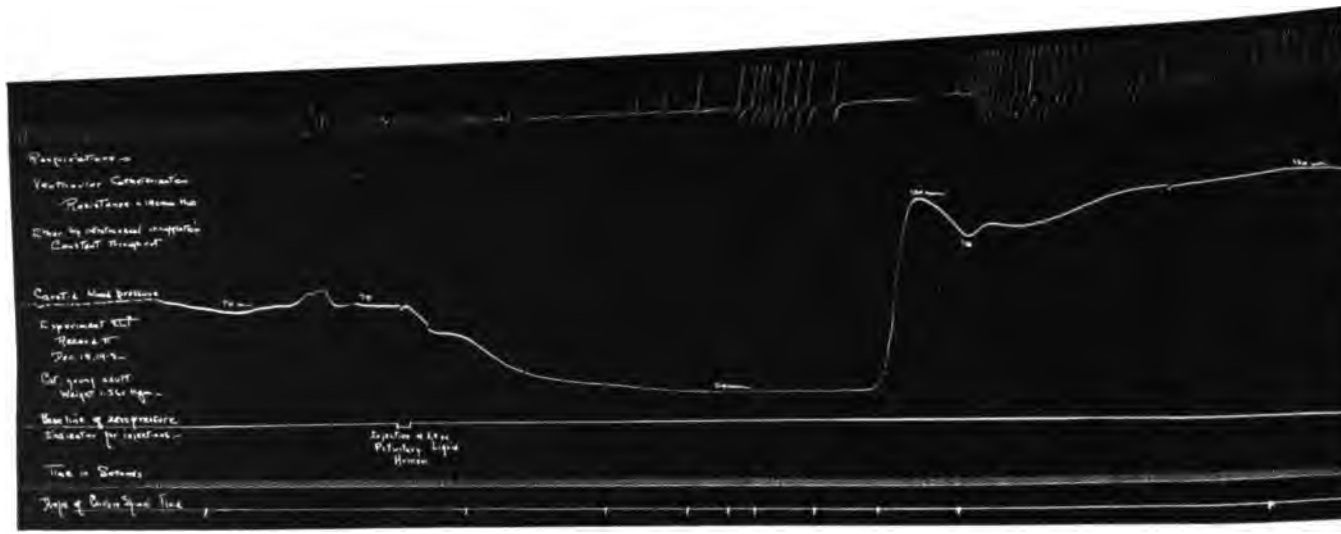
On a number of occasions we have observed an increase in the cerebro-spinal fluid outflow in association with equally extreme initial depressor responses on the part of the cardio-vascular system, but a comparison of the effects of the consecutive first and second injections (Records V and VI) of the foregoing experiment makes it seem improbable that pre-formed fluid could merely have been extruded under these conditions by intracranial venous stasis through asphyxia, for were this the case, on the restoration of free circulation one would have expected a recession of the fluid into the cannula. Such a recession, as will be pointed out later on, normally follows temporary increases in the outflow which are obviously due to mechanical causes, as for example after compression of the jugulars: and for this same reason we are inclined to attribute to physical influences the temporary outflow which accompanies the marked rise in blood pressure after injections of adrenaline.

Certain of the smaller animals, as cats and rabbits, at times appear to be extremely susceptible to the haemodynamic effects of pituitary extracts, and prolonged depressor responses may occur and prove fatal, or on the other hand the cardio-vascular readjustment may be abrupt (as in Record VI), with the exchange from the low to a high pressor phase taking place in a few seconds. An illustration of a prolonged depressor phase with recovery is shown in Record VII, giving the response in a cat to the injection of 1.0 cc. of Armour's "Pituitary Liquid." In this experiment the fluid, secured by ventricular catheterization, was dropping before the injection at the rate of a drop every two minutes, and during the 3½ minutes of the extreme depressor phase (with a blood pressure almost at zero, the pulse pressure almost nil, and nearly total respiratory cessation) the flow increased to a drop every thirty seconds. This response might possibly be attributed to asphyxiation, for the flow practically ceased after the onset of the pressor phase with its accompanying increased amplitude of respiration. However, the same response may occur (cf. Record VI) when the respiratory amplitude is increased during rather than after a profound depressor phase.

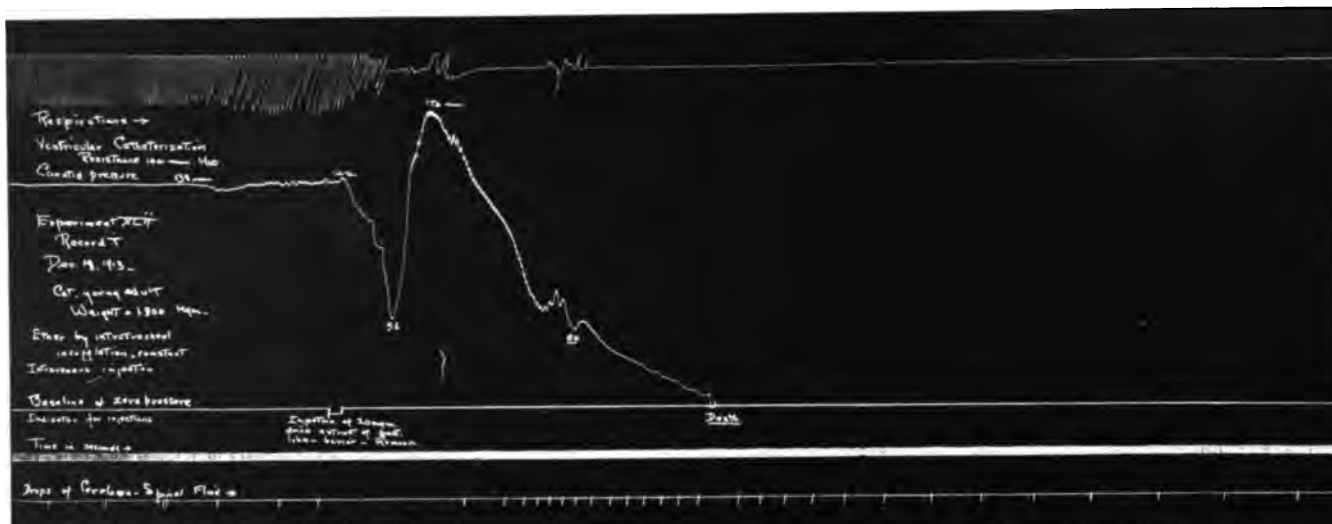
We are at a loss to account for the extraordinary phenomenon of a prolonged post-mortem outflow of fluid. This was mentioned in the protocol given above as taking place after a fatality from ether, and we have seen a number of similar responses after fatalities provoked by the injection of toxic doses of ether or of pituitary extracts, with a continued flow, which in some cases has endured for an hour after death.

An example is given in Record VIII, from a cat with ventricular catheterization. After the injection of 20 mgm. of dried extract there occurred an exaggeration of the usual vasomotor responses—a sharp fall, accompanied by respiratory cessation, then an equally sharp rise in pressure, followed in turn by a second precipitous fall to zero, with fatality. The fluid had been dropping from the catheter at the rate approximately of one drop a minute. There was a temporary cessation for 2 minutes after the injection and then, coincident with the final fall in pressure, the fluid began to flow rapidly at the rate of 4 drops a minute, until the death of the animal four minutes later, after which, gradually slowing, it continued to drop for an hour after the cessation of the circulation, 3 cc. being collected in all. Only the first 12 minutes of this remarkable response is shown in the reproduced fragment of the record.

Ordinarily after the death of an animal the fluid promptly recedes in the cannula, so that under these circumstances of a prolonged post-mortem flow some condition must serve either to expel the remaining pre-formed fluid (and 3 cc. is an abnormally large content for the ventricles of a cat) or else the fluid must continue to be produced for some time after the cessation of circulation. The response might possibly be attributed to asphyxiation, for the respiration ceased (cf. Record VIII) before the heart beat, or to an increase in tension from a post-mortem oedema of the brain. It is difficult to believe that the plexus could continue to secrete after the complete cessation of circulation, no matter what the ante-mortem stimulus might have been.



Record VII. Experiment XLI (second injection). December 18, 1913. Cat. Intratracheal anaesthesia: catheterization through aqueduct: injection of 1 cc. "Pituitary Liquid" (Armour). Showing marked depressor phase with temporary increase in fluid outflow.



Record VIII. Experiment XLII (first and fatal injection). December 19, 1913. Cat. Intratracheal anaesthesia: catheterisation through aqueduct: injection of 20 mgm. dried posterior lobe extract (Armour). Showing extreme vasomotor response with fatality and prolonged post-mortem choroidorrhoea.

V. IS THE APPARENT RESPONSE MECHANICAL OR SECRETORY?

Insufficient attention, it seems to us, has been paid to the physical conditions which underlie the production and expulsion of the fluid through the experimental cannulae. Dixon and Halliburton[19] alone appear to have subjected their results to proper standards for differentiation of mechanical and secretory effects.

The difference in tension between the capillary pressure in the choroid plexuses and that of the surrounding fluid may be termed 'the secretory pressure.' In the apparent absence of a cerebral vasomotor mechanism, or, at all events, in the absence of one which acts in conjunction with the system distributed to the body as a whole, any constriction of the splanchnic or peripheral field is followed by a cerebral hyperaemia and, according to the generally accepted view, by an increase in the intracranial tension. But within the closed cranial chamber any change in vascularity is immediately communicated to the fluid and hence as long as the chamber is intact there need necessarily be no alteration of the secretory pressure, although some of the preformed fluid, under these circumstances, is doubtless forced out through the normal channels of outflow. Hence on theoretical grounds it seems improbable that an increase in vascular pressures will cause an increased production of cerebro-spinal fluid, provided the closed system is maintained.

Quite dissimilar from this normal state are the intracranial conditions in any experiment in which a hollow tube has been introduced into some part of the fluid pathway by any method which permits decompression effects to be felt with lowering of the intracranial tension. Here the capillary resistance of the introduced cannula determines the resulting cerebro-spinal fluid pressure. The arachnoid or intraventricular tension must remain constant or at least never exceed that of the resistance established by the cannula. A rise, then, in blood pressure with a corresponding increase in capillary tension will necessarily increase the secretory pressure. Whether under these conditions

[19] Loc. Cit. Jour. Physiol. 1913, xlvii, 215.

the choroid plexus will become more permeable to the passage of fluid and thus the amount of cerebro-spinal fluid be increased is an undetermined question which presents great technical difficulties of solution. It is the generally accepted view that a probable increase of fluid production does occur under these circumstances, but the arguments are not entirely conclusive.

More important for our present considerations is the mechanical extrusion of the cerebro-spinal fluid under the influence of changes in intracranial vascularity. A cerebral hyperaemia brought about by any agency which increases blood pressure necessarily constricts the space properly filled by fluid which, under these circumstances, in normal conditions must escape by its natural outlets connected with the subarachnoid spaces, or under the experimental conditions must drop with increased frequency from the end of the cannula which leads from the ventricles.

Venous pressures likewise which are particularly under the influence of respiratory conditions unquestionably may exert considerable effect upon the secretory pressure through alterations in the capillary tension, for as Starling has demonstrated, capillary pressure follows more closely upon alterations in venous pressure than upon far greater changes in the arterial tonus. Hence, under the conditions of our experiments a marked fall in arterial pressure may be accompanied by an equivalent rise in venous tension so that the secretory pressure may remain unchanged or even be increased. Venous congestion therefore may also serve to decrease the potential fluid space, with resultant extrusion of fluid until balance be again restored. In view therefore of the possible influence of these physical factors on the discharge of cerebro-spinal fluid, it is hazardous to assume that certain agents stimulate the secretion of the choroid plexuses, merely because of an observed increase in flow from a cannula.

Were it possible to measure the cerebral capillary pressure many of these difficulties of distinguishing the agents which act mechanically from those which truly excite secretion might be solved. A convenient though less exact method of differentiation between the two processes is afforded by the employment of catheters or of cannulae which are sufficiently transparent to

allow the pulsating level of the fluid to be observed through their walls. In the course of an experiment, with the fluid dropping regularly from such a catheter, if a purely mechanical and temporary means of increasing the cerebral arterial or venous vascularity be employed, there will occur a definite and marked acceleration in the rate of flow, and very quickly the production of fluid will become constant, dependent on the new secretory balance. Then with the subsequent lowering of the congestion on the arterial or venous side as the case may be, the fluid will be observed to recede into the cannula, to compensate for the decreasing vascularity. On the other hand, if fluid is actually being secreted no such recession is observed, and though the rate may be slowed fluid continues to appear in spite of the relatively devascularized brain. This simple expedient, which Dixon and Halliburton have commented upon, of observing the recession of fluid into the cannula is oftentimes an aid in distinguishing the mechanical from the truly excitatory effects.

Another convenient check lies in the important fact that in most of these experiments the recorded drops of fluid issue from a catheter in the ventricle rather than from one inserted in a subarachnoid cistern, for as we have shown in an earlier paper, the arachnoidal villi constitute the normal channels of escape and the outflow of fluid from the subarachnoid spaces is therefore likely to be more subject to fluctuations than the outflow from the ventricles. It is quite possible, for example, that the prolonged post-mortem flow from the ventricles which we have so often observed may be less likely to occur from a cannula in the subarachnoid spaces; for ordinarily, with cessation of the circulation and resultant cerebral anaemia, the fluid immediately recedes into the catheter, being drawn upon doubtless to fill the perivascular spaces of the more or less devascularized brain. The dilatation of these spaces in the brains of animals in which arterial ligations have produced cerebral anaemia is a phenomenon which Mott was the first to point out. If these post-mortem responses are truly excitatory rather than mechanical the increased secretion apparently suffices to overcome the call for fluid on the part of the anaemic brain and to maintain the long-

continued flow of fluid, which in many cases has certainly been more than the normal ventricles could possibly have held.

Another factor which suggests that the posterior lobe preparations serve to excite secretion is the apparent fatiguability of the response on repeated injections, even though practically the same vasomotor conditions prevail. With time given for the reaccumulation of fluid one would expect a repetition of the cerebro-spinal fluid response invariably to accompany a repetition of the haemodynamic and respiratory reaction were the effect purely mechanical. We have shown that an extreme augmentation in the rate of fluid outflow is usually followed by an abrupt and persisting cessation of the flow[20] (e.g. Records II and IV): in other instances that a cessation of the flow occurs but is more gradual in onset (e.g. Record III): in still others the flow though slowed may continue after its temporary augmentation (e.g. Record I). In some animals after a prolonged cessation the flow may be incited by a second and even by several subsequent injections (e.g. Protocol of Experiment LIV), but after one outspoken reaction a subsequent response to pituitary preparations is apt to be much less striking even though an abundant flow may be obtained by the injection of other substances, ether seeming to be especially potent in this respect. It would appear that the fatigue threshold of the choroid plexuses is much lower to pituitary extracts than is that of the vasomotor system for the same substances. It is possible too that the choroid cells may in some cases respond to smaller doses than those required for the typical vasomotor reaction, for in a few instances in which the amount of the extract has failed to provoke any apparent change in the blood pressure a brief but definite increase in the rate of the outflow of cerebro-spinal fluid has occurred.

[20] It may again be emphasized in this connection that in the case of a permanent cessation of the flow after an outspoken primary response subsequent injections of an active preparation, which calls out anew a pronounced blood pressure response, may serve to express a few drops of fluid from the cannula followed by an immediate recession of the fluid. This we regard as purely a mechanical effect.

VI. RECAPITULATION AND CONCLUSION

Extracts of the posterior lobe of the hypophysis introduced intravenously[21] serve to discharge cerebro-spinal fluid from a calibrated catheter introduced in its pathway (ventricle or subarachnoid cistern). Positive responses occur to the injection of nearly all the fluid proprietary preparations, though in our hands the desiccated extracts have proved to be the more active. The possible increment to the fluid through increased permeability of the cerebral capillaries and drainage out along the perivascular lymphatics is excluded in most of these experiments by the method of tapping the fluid pathway in the third ventricle rather than in the cisterna magna. The response is seen to occur under most variable conditions and appears to be independent of respiratory influences or haemodynamic reactions, for it may coincide with periods of respiratory cessation or acceleration or with periods of arterial hypertension or depression. A prolonged flow from the ventricle may continue even after death and may reach an amount apparently in excess of the normal content of the ventricles. That there is an actual increase in the amount of fluid secretion is shown by its non-recession into the cannula following periods of increased cerebral vascularity as well as by the measurement of the amounts discharged. The evidence inclines us to believe that the outflow under the circumstances of our experiments represents an actual secretory response rather than an expulsion of pre-formed fluid due to physical conditions resulting in changes in the volume of the brain.

Hence the conclusion may be justified that:

Extracts of the posterior lobe of the hypophysis increase the rate of production of cerebro-spinal fluid (choroidorrhoea) by stimulating the secretory activity of the choroid plexuses.

[21] In a few experiments we have attempted, without success, to excite choroid secretion by stimulating the superior cervical ganglion, in the expectation of discharging the hypophysial secretion in this way (cf. Weed, Cushing and Jacobson: The Autonomic Control of the Pituitary Body. Bull. Johns Hopkins Hosp., 1913, xxiv, 40–52) and also by injecting extracts into the ventricle itself on the view that the presence of posterior lobe secretion in the fluid itself as well as in the circulation may excite secretion (cf. Cushing and Goetsch: The Secretion of the Posterior Lobe of the Pituitary Body and its Presence in the Cerebro-spinal Fluid. Amer. Jour. Physiol., 1910, xxvii, 60–86.)

THE BLOOD PRESSURE DURING VOMITING

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The present work is a continuation of the studies of the blood pressure and its variations in the unanesthetized animal under different normal and pathological conditions. The methods adopted for taking the blood pressure are those previously described.[1]

The object of the present research was to study the blood pressure preceding, during, and following the act of vomiting; and to ascertain as far as possible the mechanism of the changes in the blood pressure or the regulation and control of the blood pressure at such a time.

Before this work was begun, we assumed that during vomiting the blood pressure was greatly heightened. This assumption appears to be generally prevalent. Text-books as a rule do not mention blood pressure changes during vomiting. In those text-books in which it is mentioned, there is no statement of facts or experimental work done; but merely indirect statements inferring that the blood pressure is greatly augmented during vomiting.

Clinical literature contains a good many indirect references to this subject and physicians are warned not to give emetics to patients whose arteries are atheromatous lest there be a rupture of the blood vessels.

The first tracing taken by us was during a study on the unanesthetized dog under the action of digitalis (with Dr. H. G.

A preliminary report of this work was made before the American Physiological Society at Cleveland, December, 1912.

[1] Brooks, C., Heart, 1910, vol. 2, p. 5.

Schleiter). It showed that the blood pressure, instead of rising during vomiting fell rather sharply to a low point and after the act of vomiting, rose rapidly to the normal or slightly above the normal. This was followed by a series of experiments carried on in the Hull Laboratory of Physiology, University of Chicago and later other experiments at the Laboratories of Physiology and Pharmacology at the University of Pittsburgh.

Six months after our paper had been presented at Cleveland[2] while attending the Minneapolis meeting of the American Medical Association, June, 1913, rumors were heard of older literature on the subject of the blood pressure during vomiting. Dr. Cannon referred us to Dr. Gatch, who he said, had found an important reference to this subject. Dr. Gatch himself stated that his idea was obtained from a reference in Hall's Text Book of Physiology. Dr. Hall writes, in part: "Traube was the first to show that at the commencement of vomiting typical vagus pulsations occur, resulting in a lowering of the general arterial pressure. At the termination of the attack the pulse becomes accelerated and the arterial pressure rises even above the normal."[3]

About this time (June, 1913) an article by Hesse[4] directed our attention to a remarkable volume by Guinard[5] on the subject of morphine and apomorphine in which we found descriptions of experiments and tracings the results of which are in complete accord with those reported in our preliminary report.

[2] An abstract of the paper prepared for publication in the Proceedings of the American Physiological Society of that year (1912) was refused publication because of its length. A shorter abstract reached the editor of the American Journal of Physiology too late for publication.

[3] A Text Book of Physiology, Winfield S. Hall, Lea Brothers & Co., 1905, pp. 411–412. The reference to Traube's work given at the end of the chapter is most inaccurate (Pflüg. Arch., vol. iv, p. 131). Traube's name does not appear as one of the contributors of the first 124 volumes (1908) of Pflüger's Archiv. We have not, after days of search assisted by a most able librarian, been able to find any article by Traube on the subject of vomiting, whether in Pflüger's Archiv or in any other scientific journal.

[4] O. Hesse: Zur Kenntnis des Brechaktes. Arch. f. gesam. Physiol., vol. 152, June, 1913, pp. 1–22. Hesse reprints one of Guinard's tracings.

[5] Louis Guinard: Étude Experimentale de Pharmacodynamie comparée sur la Morphine et l'Apomorphine. (These) Faculté de Médecin et de Pharmacie de Lyon, Tome 10, 1897–1898.

The tracings in this article are as nearly identical with some of our own as blood pressure tracings could well be. To Guinard, therefore, belongs the credit for priority in this field. We are sorry that we failed to discover his work sooner; but since his research was a thesis not published in any accessible journal it escaped notice. In this paper we wish to call attention to this work confirmed by us, and to extend the work by adding experiments which throw some light on the mechanism which is responsible for the results obtained by him and ourselves.

Though E. Harnack[6] many years before Guinard purported to make a study of blood pressure and pulse rate during vomiting induced by apomorphine an examination of his protocol-tables shows that at the exact moment of emesis and for some minutes afterward (7–20 min.) he took no record of the blood pressure or the pulse rate. As a result he failed to discover the remarkable phenomenon first described by Guinard and confirmed by our work. His experiments deal with blood pressure phenomena preceding and following the injection of apomorphine but he has no data on the blood pressure and pulse rate at the moment of expulsion of the vomitus.

EXPERIMENTAL METHODS

The methods employed in these experiments are those previously devised for study of blood pressure on the quiescent unanesthetized animal.

The T-cannula was inserted into the carotid under ether anaesthesia and several hours later or the following day, when the animal had recovered from the effects of the anaesthetic, the studies on the blood pressure during vomiting were made. Several additions have been made in the technique used lately. These briefly described are as follows:

First is the tambour piston separation cannula. This instrument is a modification of the old T-glass cannula. It consists of a metal cannula whose side arm is relatively of large calibre

[6] E. Harnack: Arch. f. exp. Path. und Pharm., vol. 12, 1874, p. 254. See tables on pp. 261, 262, and 263.

and contains a piston consisting of a hollow hard rubber cylinder with a thin rubber diaphram over one end. This rubber diaphram can give way and thus permit the column of liquid to transmit the impulses from the heart to the mercury manometer with slight damping of the curve. For the larger changes in the blood pressure the piston itself can slide up or down following the pull of the rubber diaphram. The advantage of the diaphram separation cannula is, that it separates practically completely the blood from the fluid in the manometer tube and therefore prevents the entrance of toxic substances into the circulation which constitutes a difficulty in work done upon the quiescent animal. The second is an oil separation cannula. The object of the instrument is the same as that of the rubber diaphram separation cannula; but here the separation is accomplished by means of an inverted U-tube filled with oil which floats upon the blood column in one arm, and the fluid which is used to connect with the mercury manometer of the other arm. This accomplishes the separation without the interposition of any diaphram and is therefore without the slight damping effect observed in the diaphram cannula.

A third addition to the technique is the injection of a sufficient amount of leech extract into the cannula at intervals to prevent clotting. The leech extract is prepared by the method of Abel, Rowntree and Turner,[7] or by grinding up the heads of leeches in a mortar, extracting for 24 hours in water, precipitating the proteins by heat and dilute acetic acid and filtering and neutralizing with sodium bicarbonate.

The experiments were performed upon dogs. Vomiting was induced by digitalis, apomorphine subcutaneously, and by zinc sulphate, magnesium sulphate by the mouth. Different types of animals were employed for different experiments; for example, small wiry muscular fox terriers were employed for studying the effects of the muscular contractions of the abdomen and other skeletal muscles concerned in vomiting, and on the other hand

[7] Abel, Rowntree and Turner: On the Removal of Diffusible Substances from the Circulating Blood of Living Animals by Dialysis. J. Pharm. and Exp. Therap., vol. v., no. 3, Jan., 1914, pp. 275–316.

young adults or half-grown pups with large abdomens were used to study those phenomena of vomiting that are brought about by the gastric contraction. Observations on the respiratory movements were made by inspection and in some instances by tracing the movements by means of a tambour.

EXPERIMENTAL RESULTS

Our results indicate that there are different types of vomiting, the variations depend principally upon the character of the drug employed, or upon the consistency of the gastric contents or the degree of participation of the skeletal musculature or of the diaphragm or the gastrointestinal musculature respectively.

In general there are two types of vomiting.

First there is the rapid projectile type, characterized by suddenness of onset, with slight premonitory symptoms, and with apparently a preponderance of the gastro-intestinal tract activity over the activity of the skeletal muscular system.

Second there is the slow labored type of vomiting characterized by rather marked prolonged premonitory symptoms and with apparently more of the skeletal muscles taking part in the process which consists of prolonged violent retching movements, meanwhile there are great oscillations of the blood pressure, and which ends with vomiting followed by exspiration and a noise just after the vomitus is expelled. Or to summarize:

First type. Rapid projectile. Preliminary nausea with slight rise in blood pressure and with a decrease in amplitude of pulse wave, and with an increase in pulse rate. Vomiting is accompanied by a sharp fall in the blood pressure, during which there is slowing or almost cessation of the heart beat, or arrhythmia. Respiration is almost completely suspended so far as ventilation of the lungs is concerned for the epiglottis is closed The vomitus is expelled near the low point of the blood pressure curve (fig. 1) and immediately after there is a return of respiration and a recovery of the blood pressure which rapidly mounts to the normal or even above the normal.[6]

[6] Dr. A. J. Carlson observed a drop in blood pressure and slowing of heart in an unanesthetized dog when vomiting was induced by the inhalation of ozone.

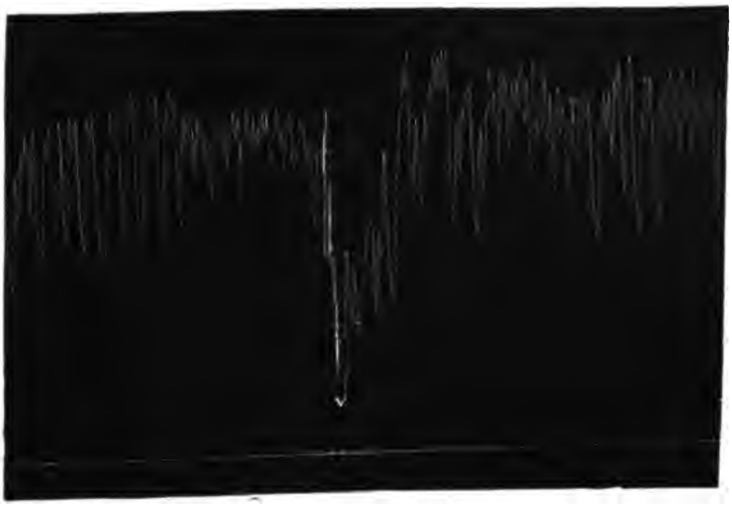

Fig. 1. Blood-pressure tracing showing fall in blood-pressure during vomiting. The vomiting is of the quick projectile type. "V" shows the point where the vomitus was thrown out. Time is recorded in seconds.

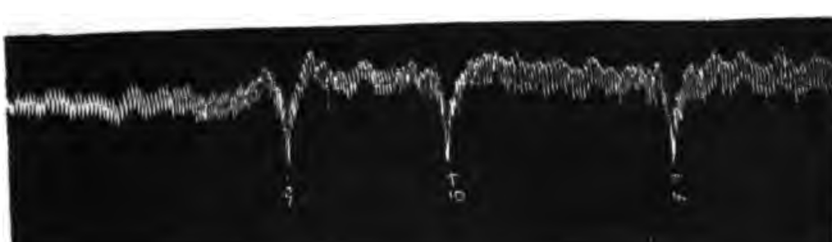

Fig. 2. Type of tracing obtained during quick projectile vomiting where atropine had been previously administered. Vomitus was expelled at 9, 10, and 11.

Second type. Slow labored type. Preliminary nausea, deep breathing, salivation. Then comes on violent retching with great oscillations of blood pressure. At this time the pulse beats are irregular and slow. Respiration is suspended so far as the ventilation of the lungs is concerned but there are violent changes in the intrathoracic pressure which correspond with the retching movements. After a long series of retching movements, the vomitus comes up into the pharynx at the instant the top of one of the violent blood pressure oscillations is reached (fig. 3). The animal then takes breath and the blood pressure after suddenly falling again during the inspiration mounts to the normal or above the normal.

Vomiting after the administration of atropine

It was thought that since the vagus center and the central nervous areas that are concerned in vomiting, are adjacently located in the central nervous system, that it was very probable that the fall in blood pressure and the slowing and the irregularities in the pulse rate during vomiting are brought about through the vagus mechanism. Evidence in favor of this view was obtained by first making the observation of vomiting on the normal dog, and then later on the same dog, making a second observation of vomiting after the administration of atropine. It was found that after the administration of sufficient atropine to paralyze the vagus endings of the heart, that the vomiting failed to produce the marked fall in blood pressure, the amount of slowing of the heart, nor the degree of arrhythmia, as had been observed in the same animal previous to the administration of the atropine (fig. 2).

Vomiting after the administration of curare

It was thought that the great oscillations of blood pressure noticed during the violent retching movements seen in the second type of vomiting were due chiefly to the action of the skeletal muscles in the violent contractions, as well as their effects by pressure on the abdomen and vacuum formation in the thorax.

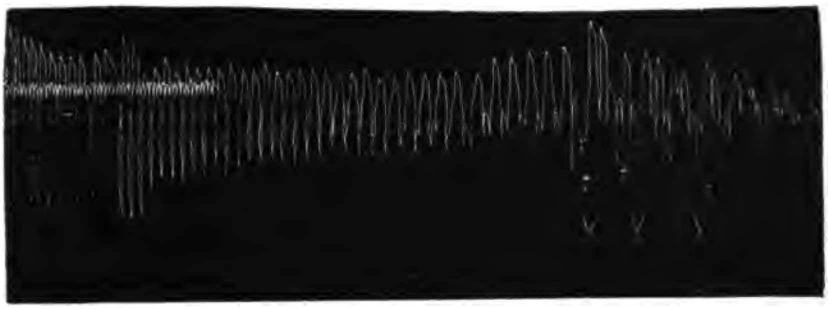

Fig. 3. Blood-pressure tracing during vomiting of the slow labored type. Here violent retchings continue for a long time. The tracing begins several seconds after the onset of retching. At the points marked "V" vomitus was expelled and some breath taken. At the left end of the chart the second round of the blood-pressure tracing is lapped for a few inches in order to show the height of the pressure as compared with that of the first round which was taken during the retching period.

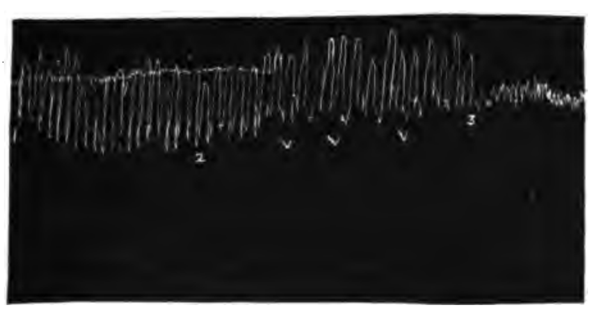

Fig. 4. Blood-pressure tracing showing a reduction of retching movements in the slow labored type of vomiting. This reduction was caused by a small dose of curare. On the left end of the chart the second round of the tracing continued for a short distance lapped over the first round in order to show the comparative height of pressure during the retching period and shortly after vomiting. 1, 2, retching. V, vomiting. 3, recovery.

Therefore it was further thought that if this be true, then after more or less complete paralysis of these skeletal muscles by means of curare, vomiting should show a corresponding decrease in these osci lations of the blood pressure.

It was found that after the subcutaneous administration of curare in sufficient quantities to have a marked depressing effect upon the animal, but not sufficient to cause complete immobility then vomiting failed to produce the violent retching, and likewise failed to produce the great oscillations of the blood pressure (fig. 4). The conception of the mechanism involved is as follows:

1. Vagus inhibition of the heart is an important factor in the production of the lowering of the blood pressure, inhibition of the hea t, and arrhythmia noticed during vomiting. In an animal in which the cardio-inhibitory nerve endings have been paralysed with atropine these blood pressure changes just mentioned are reduced.

2. Muscular contraction *per se* causes an increase in the blood pressure. The violent contractions of the skeletal muscles seen during retching always cause an increase in the blood pressure, and there is a fall in the blood pressure during the periods of relaxation which intervene. This rise in the general blood pressure observed during retching can be diminished by partly curarizing the animal.

SUMMARY

Vomiting is accompanied by marked changes in the circulatory system and interference with respiration. There is seen sometimes a period of elevated pressure, more frequently a sudden and enormous drop in blood pressure with cardiac inhibition at the moment of emesis, always periods of great oscillations in the blood pressure. These great and sudden oscillations of the blood pressure may cause a rupture of the blood vessels which would not occur with the same pressure but with slower changes. The faint feeling accompanying emesis may be the result of the cerebral anemia occasioned by the cardio-inhibition and resulting drop in blood pressure.

THE EFFECT OF THYROID EXTRACTS UPON BLOOD PRESSURE[1]

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The function of the thyroid gland is probably dependent upon the production of certain substances constituting an internal secretion which reaches the tissues through the medium of the blood and lymph. It is reasonably certain that the active portions of this secretion can be obtained from the gland by various methods of extraction. The effects upon the blood pressure of extracts from the various glands of internal secretion have been tested in numerous laboratories during the last twenty years.

The first kymograph experiments to determine the effect of intravenous injections of aqueous extracts of the thyroid were made by Oliver and Schafer (1) who obtained a marked fall in blood pressure. They found that extracts of the fresh glands were somewhat more active than those made from old or dried material. A glycerin extract was no more effective than the aqueous preparation. Boiling the extract did not alter its depressant effect which apparently resulted from its action upon the heart. Oliver and Schafer found also a similar blood pressure depressing effect to follow the intravenous injection of extracts made from many other organs.

Haskovec (2) experimented with extracts of the thyroid similarly prepared and in the main confirmed the previous findings. He, however, obtained a slightly increased frequency of the pulse rate which seemed to be due to central stimulation of the cardio accelerator nerve. He attributed the fall in blood pressure to a

[1] The expenses of this investigation were defrayed by the Johnston Livingston fund for Experimental Therapeutics.

direct action upon the heart muscle. He experimented also with alcoholic extracts of the thyroid gland and found that intravenous injection of these extracts produced no change of blood pressure.

Georgiewsky (3) experimented with thyroid extracts prepared in a similar manner and found essentially the same results but Guinard and Martin obtained a retardation instead of acceleration of the pulse rate.

Fennyvessy (4) found the fall in pressure to occur after section of the vagus and after the administration of atropin and considered it due to a peripheral effect of the extract.

Svehla (5) could obtain no effect from the injection of extracts of embryonic thyroids and only slight effect from those derived from the glands of new born animals.

Several other investigators contributed confirmations of the results of these last two investigators.

Heirratz (6) and Livon (7) claimed from their experiments with aqueous extracts of the thyroid an elevation of blood pressure.

More recently Asher and Flack (8) injected intravenously measured quantities of alkaline aqueous extracts of fresh and dried thyroids and could detect little or no effect upon the blood pressure.

In contradiction to these results Popielski in 1911 (9) isolated from various organs, including the thyroid, a substance which he called vasodilatin which substance he claims is in no way identical with choline or β-imidazolylethylamine and is the active principle of organ extracts. This substance was obtained by digesting the proteins of the glands with pepsin and trypsin. Details of the method are not given. Popielski claims that his vasodilatin diminishes blood pressure, produces incoagulability of the blood and favors the secretory activity of the glands. This diminished blood pressure is not brought about by change in heart action but by direct dilatation of the blood vessels.

Lampe, Liesegang and Klose (10) experimented with alkaline extracts of human thyroid, both normal and goitrous, and obtained only a slight fall in blood pressure without acceleration

of the pulse. But extracts of the fresh thyroids obtained from operated cases of Graves disease injected into nervous fox terriers produced a fall in blood pressure of 20 to 40 mm. of mercury, accelerated the heart action and raised the temperature. A repetition of their experiments by O. Bardenhewer (11), however, failed to confirm these findings.

It is interesting to note, however, in conjunction with the seemingly constant presence of a vasodilator substance in normal thyroids and the probable excess of thyroid secretion in conditions of hyperthyroidism, some recent experiments of Black and Sanford (12). They obtained serum from patients suffering from more or less pronounced Graves disease, and found after its intravenous injection into dogs a drop in blood pressure which corresponded in some degree to the severity of the process in the patient from whom the serum was obtained. Control experiments with serum from nervous subjects without evidence of hyperthyroidism were negative. Extracts made from diseased thyroids not of the exophthalmic goitre type removed by operation produced some fall in blood pressure but it was not as marked as the fall obtained from extracts of the glands of pronounced Graves disease. They state that a repetition of the injections in the same animal seemed to establish a tolerance. The second and third injections during the usual experimental period produced less effect than the first.

V. Fürth and Schwartz (13) have attempted to prove that the vasodilator effects which have been noted after the injection of extracts of practically all organs and tissues are due to the presence in them of the same substance; namely, cholin. This conclusion is very probably erroneous, as shown by Vincent (14).

It is evident that aqueous extracts of the thyroid may contain more than one active substance. Frankel (15) extracted the gland in boiling water and after removing the albumen obtained a crystalline body, having the formula $C_7H_{11}N_3O_2$. But this contains no iodin, and though it was said to have produced hyperthyroid symptoms after subcutaneous injection, no kymograph tracings were made.

Baumann (16) produced so called iodothyrin by boiling the

thyroid in 10 per cent sulphuric acid. This substance contains a high percentage of iodin and is used therapeutically but numberless experiments have proved that at least it is not the normal active ingredient of the organ.

Oswald (17) has attempted to show that iodothyrin is derived from a preceding thyroglobulin in the colloid. All investigators are practically agreed that whatever the active ingredient of the thyroid may be, it has a definite relation to the content of iodin.

A comparison of the results of injections of thyroid extracts and of iodothyrin into the circulation shows quite marked differences with different observers but the weight of evidence is against any noticeable fall of blood pressure after intravenous injections of iodothyrin. It, nevertheless, seems to show rather constant effects upon the nerve supply of the heart, the details of which appear to vary in different animals. von Cyon and Oswald (18) state that it stimulates the cardio-inhibitory center and increases the electric excitability of the vagus and affects the terminal filaments in the heart muscle. von Fürth and Schwartz (13) claim that it has a direct effect upon the heart muscle. Barbera (19) compares these results with injections of iodin and its salts, and seems to show that the latter have an antagonistic action to iodothyrin. Iodin and its salts apparently paralyzed the vagus and its nervus depressor, and excited the cardiac and vascular sympathetic nervous system. After injections into the circulation of iodothyrin the electric irritability of the vagus and sympathetic nerves is increased. The salts of iodin have an opposite effect, but seem to directly stimulate the sympathetic and produce in dogs and rabbits an increase in temperature, blood pressure and pulse rate. We have not been able to confirm these findings. von Cyon (20) has attempted to prove that the electric irritability of the nervus depressor after the injection of iodothyrin is greatly increased. Asher and Frank (21) have assumed that von Cyon's statements are correct and that the secretory nerve fibres for the thyroid are derived from the superior and recurrent laryngeals, and claim that electric stimulation of the nervus depressor shows much more pronounced effects after than before a preceding stimulation of the laryngeals. In other words, that an increased output of thyroid

secretion produced by stimulation of the secretory nerve supply of the gland, sensitizes the nervus depressor. If the thyroid is removed and the laryngeals then stimulated, no increased excitability in the nervus depressor is demonstrable. They also state that after stimulation of the laryngeals, which is supposed to produce an increase in the thyroid output, an injection of adrenalin produces a higher rise in glood pressure than a similar injection made when the laryngeals have not been stimulated.

Kraus and Friedenthal (22) state that mixing adrenalin with extracts of thyroid prolongs and intensifies the action of adrenalin.

From the experiments quoted it is quite definitely established that extracts of the entire thyroid gland contain a substance or substances which have a pronounced effect on lowering blood pressure. These effects are more pronounced when obtained from fresh rather than dried glands and from those from mature rather than young animals. It is also clear that boiling does not destroy the vasodilator property. The effects upon pulse rate and respiration are at least insignificant.

Our experiments have been begun with an attempt to isolate from a watery extract of the fresh gland all of the substances which can be separated with as little alteration as possible of their chemical structure. These include the socalled nucleoproteins, globulins, coagulable and non-coagulable albumens, and the alcohol soluble and alcohol insoluble residue. Each of these bodies has been dissolved and injected intravenously in dogs, and the results upon the blood pressure, respiration and heart action noted in the usual kymograph tracings. The solution of the alcohol-soluble residue left after removal of all other substances has been found to be the only substance which produces any appreciable effect upon the blood pressure.

The glands used throughout these experiments have been pig thyroids. These were chosen because of their relatively high iodine content over those of the beef or sheep. It has also been found that the separation of the nucleoproteins from the extract of the pig glands was readily brought by precipitation with acetic acid without warming to 44°C. as required by the extracts from sheep and beef glands.

PREPARATION OF THE VARIOUS THYROID EXTRACTS USED IN THE EXPERIMENTS

The thyroid glands were obtained from a slaughter house in as fresh a condition as possible. They were trimmed free from any adhering fat or connective tissue and immediately finely ground up in an ordinary meat grinder. This hashed up material was then placed in large, wide-mouthed bottles with twice its volume of physiological salt solution made faintly alkaline with sodium hydroxide. It was shaken for two hours and then placed in a refrigerator over night.

After this extraction the liquid was filtered off, first through a double thickness of cheesecloth and then through paper pulp in a Büchner funnel. The extract was now ready for separation.

I. The nucleoproteins were precipitated by acidifying with 10 per cent acetic acid. After standing for half an hour or so they settled out and were filtered off. The proteid precipitate was ground up, suspended in distilled water, dissolved with 10 per cent sodium hydroxide, filtered, and reprecipitated with 10 per cent acetic acid. After settling out the nucleoproteins were washed by decantation five or six times with distilled water. After being again suspended in water and dissolved with as little 10 per cent sodium hydroxide as possible, they were ready for use.

The acid filtrate from the first precipitation was the basis of all the remaining proteins used.

II. The globulins were obtained by half saturation of this acid filtrate with ammonium sulphate. These were filtered off, dissolved in distilled water, placed in parchment paper dialysing bags and dialysed for ten days or until there was no sulphate shown to be present.

III. The filtrate from the globulins was saturated with ammonium sulphate and the albumins so obtained were filtered off and dialysed exactly as the globulins.

IV. The coagulable proteins were obtained by boiling the acid filtrate, filtering off the coagulated portion, making faintly alkaline with sodium hydroxide, boiling again and again filtering.

The combined precipitates so obtained were washed several times with distilled water and then dissolved with as little sodium hydroxide as possible.

V. The protein substance that is here called the "residue" was the filtrate remaining after all the coagulable proteins had been removed. This was always used in a slightly alkaline condition.

VI. The residue was evaporated to as near dryness as possible and found to be partly soluble in 95 per cent alcohol. This gave the two preparations called "alcohol soluble" and "alcohol insoluble." The alcoholic solution was evaporated to drive off the alcohol and the residue remaining was taken up with distilled water in which it was readily soluble, and made just alkaline with sodium hydroxide. The alcohol insoluble portion was dissolved in water and made faintly alkaline in the same manner.

VII. The portion "alcohol soluble" was further fractionated by completely precipitating the peptones with basic lead acetate. This gave the two portions called "lead precipitate" and "lead filtrate."

The lead precipitate was washed several times with distilled water to free it of the filtrate, made slightly acid with acetic acid, heated and decomposed by passing sulphuretted hydrogen gas through it. After complete precipitation of the resulting lead sulphide, it was filtered and the filtrate evaporated almost to dryness, taken up again with distilled water, filtered and made faintly alkaline before using.

The lead filtrate was treated similarly with sulphuretted hydrogen gas to precipitate any excess of the basic lead acetate, filtered, evaporated to approximate dryness, taken up with distilled water, filtered again and made faintly alkaline for use.

VIII. Some iodothyrin according to Baumann's process was obtained and a saline extract of this prepared for comparative work as was also physiological salt solution extract of some "Desiccated Thyroid Glands" prepared by Parke, Davis & Co.

The protein content of each of these preparations was obtained by finding the per cent of nitrogen by the Kjeldahl method and multiplying this by the factor 6.25. The iodine content was

obtained by Rigg's modification of the Baumann process with the further slight modifications given in a previous paper. Whenever two or more of the various preparations were made from different sets of glands, the ratio of the nitrogen and iodine in each was found to be fairly constant and any variation can be accounted for under seasonal variation if not merely within the limits of unavoidable technical errors.

Further work in regard to the chemical nature and properties of this "residue" is being carried on and will be published in subsequent papers.

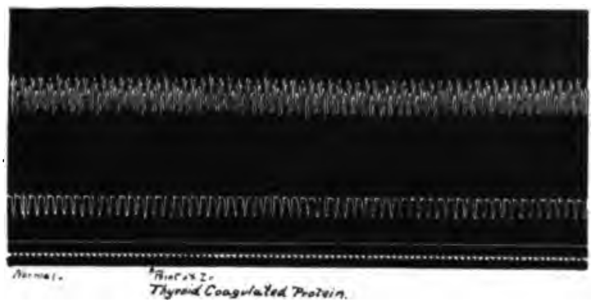
The following table shows the nitrogen and iodine content of the extracts used:

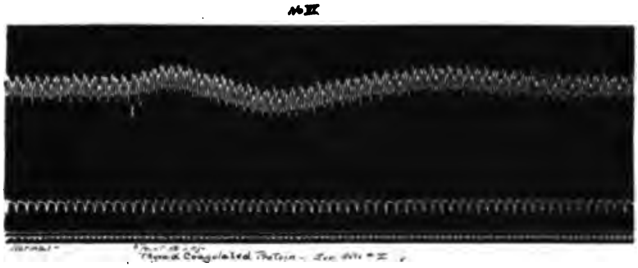
	PER CENT OF PROTEIN	MGS. OF IODINE PER CC.
Nucleoproteins	1.860	0.12000
Globulins	0.700	0.01360
Albumins	0.175	Trace
Coagulable Proteins	0.800	0.01140
"Residue"	4.025	0.02785
Alcohol Soluble	5.820	0.00880
Alcohol Insoluble	2.700	0.01040
Lead Precipitate	0.660	Trace
Lead Filtrate	2.660	0.00050
Iodothyrin	0.407	0.00015
Parke, Davis & Co., desiccated thyroid	0.500	0.02400

The proteins described in the previous pages were used in an extensive series of experiments to determine their effect upon blood pressure, pulse rate and respiration. In the preliminary experiments we were struck with the marked fall in blood pressure produced by the non-coagulable portion of the extract called "Thyroid Residue." This was so marked in its effect that we were led to compare all the other fractions to it giving comparable doses in each instance. It was further found that the depressant substance was located in the alcohol soluble portion of the residue; also in the filtrate from the lead precipitate. It was further demonstrated that the depressant effect of the various proteins bore no relation to the content of nitrogen and iodin.

Dogs were the experimental animals used throughout the experiments. The injections were all made in the femoral vein, blood pressure taken from the carotid artery. Before the injections were begun numerous experiments were made to determine the effect of anesthesia on the blood pressure and it

No. III





was found possible to keep the dogs under ether for periods of three or four hours without changes in the blood pressure.

In striking contrast to the effect obtained by intravenous injection are the results showing that the subcutaneous injection of enormous doses of the most active preparation obtained produced no effect upon the blood pressure. The experiments were varied in some instances, giving comparable doses of nitrogen and in other instances comparable doses of iodin but the results

were absolutely uniform in showing that the complete proteins did not produce depressing effects in any way comparable to the effects produced by the alcohol soluble and lead filtrate portions of the residue. Enormous doses of the protein produced only relatively slight reactions. The intravenous injection of the active portions of the residue never failed to produce a reaction when the blood pressure in any degree approached normal. After the injection of large doses of the proteins without effect, a small dose of the residue produced a prompt response.

In all experiments were made upon seventy-five dogs. The tracings which are published were taken from three animals but they are identical with those obtained in a large series. The recovery of the animal following a small injection of the active portion of the residue is prompt as will be seen from the tracings. The injection of large doses of residue caused a depression in blood pressure nearly to the zero line; the recovery to normal in such cases was very gradual but finally complete.

On account of the limited space, only three of the typical tracings will be produced. Tracing No. III, showing the effects of the Thyroid Coagulated protein, with its protein dosage equivalent to that of the Residue, in tracing No. VIII, and tracing No. VI with its protein and iodine dose many times stronger than that of the Residue. Tracing No. VIII is typical of the effects of the Residue. The effects of the remaining preparations from the Thyroid are given in Plates I and II in tabulated form. The remaining tracings are filed with this JOURNAL.

SUMMARY

1. Iodine is present in all the fractions of the extract.
2. The coagulable fractions, which are the richest of all the fractions in iodin, produce little or no depressor effect even in relatively enormous dosage.
3. The "Residue," or fraction of the extract which remains after the removal of the coagulable fractions, contains iodin but in smaller quantity than the other fractions.
4. The depressor effect of the "Residue" bears no relation to the total iodin content of the gland from which the residue is derived.

PLATE I

	Tracing Number	I	II	III	IV	V Note #I	VI Note #II	VII Note #III	VIII Note #IV	IX Note #V	X	XI Note #VI	XII Note #VII
	Thyroid Solution	Residue	Globulin	Coagulated Protein	Albumin	Globulin	Coagulated Protein	Albumin	Residue	Residue	Nucleo-protein	Thyreo-iodin	Residue
Protein	Mgms. per c.c. Solution	40.25	7.	8.05	1.75	7.	8.05	1.75	40.25	40.25	18.6	4.07	20.
Iodine	Mgms. per c.c. Solution	.02785	.0136	.0114	Trace	.0136	.0114	Trace	.02785	.02785	.12	.00015	.0185
Dosage Intra-venous.	Minims	5.	26.3	22.9	105.6	263.	320.	528.	5.	5.	10.	240.	8.6
	Mgms. Protein	11.55	11.55	11.55	11.55	115.5	167.	57.75	11.55	11.55	11.55	183.	11.55
	Mgms. Iodine	.0087	.02235	.016316	Trace	.2235	.2274	Trace	.0087	.0087	.075	.00675	.0106
Effects on Blood Pressure	Fall in Mms. Mercury	32.	0.	0	0	Secondary Fall 3 mms.	Second-Fall. 10 mms.	Second-Fall. 12 mms	38.	49.	0	0	22.
	Rise in Mms. Mercury	0.	0.	0	6.	Primary Rise 4 mms	Primary Rise 5. mms	Primary Rise 6 mm	0	0	0	0	0
	Latent Period before Effect.	4.sec.	0.	0	13.sec.	2.sec.	2.sec.	27.sec	9.sec.	4.sec.	0	0	4.sec.
Effects on Heart Rate	Rate per min. before injection	144	150	138	138.	141	144	144	138.	205	198	198	216
	Rate during time of Action	144	—	—	138.	141	144	144	See Note #VIII	204	—	—	210
	Rate after Bld. P. returned to Normal	144	150	138	138	141	144	144	138	204	198	198	210
Time of Action		2 min.	0	0	2 min. 47 sec.	40 sec.	1 min. 20 sec	2 min.	2 min. 49 sec.	1 min 44 sec	0	0	49 Sec.
Respiratory Effects		0	0	0	0	0	0	0	0	0	0	0	0

Notes and Remarks.

#I Ten times the protein dose and 25.5 times the Iodine dose of Residue.
#II 14.5 " " " " " 26.1 " "
#III 5. " " " " " Iodine was present only in slight trace.
#IV Residue inj. to check Bld. P. condition on dog #1.
#V New dog. #II.
#VI This is the Thyreoiodin prepared according to "Baumann".
#VII Third dog, and new solution of Residue. Protein dose same as in Tracing #I Iodine .0019 mgms greater.
#VIII At lowest point of curve of Bld. P. Pulse rate 126 per min. at middle of rising Bld. P. Pulse rate 132 per min.

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PLATE II

	Tracing-Number	XIII Note #VIII	XIV Note #IX	XV Note #X	XVI Note #XI	XVII Note #XII	XVIII	XIX[1]	XIX[2]	XIX[3]	XIX[4]	XX
	Thyroid Solution	Residue Alcohol Soluble	Residue Alcohol Insoluble	Residue Pb Filtrate	Residue Pb Ppte.	Parke-Davis Dessicated Thyroid	Same as XVII	Residue Note #XIII				Residue Note #XIV
Protein	Mgms. Per c.c. Solution	.58.2	27.	26.6	5.	5.	5.	20.				20.
Iodine	Mgms. Per c.c. Solution	.0088	.0104	.0005	Trace	.024	.024	.0185				.0185
Dosage Intra-Venous	Minims	18.	64.	6.3	160.	34.65	160.	160.				8.6
	Mgms. Protein	69.8	115.5	11.55	53.3	11.55	53.3	213.				11.55
	Mgms. Iodine	.0106	.0443	.00021	Trace	.0561	.256	.1973				.00106
Effects on Blood Pressure	Fall in Mms. Mercury	40.	12.	19.	18.	0.	12.	83.				40.
	Rise in Mms. Mercury	Secondary 12.	0	0	0	0	0	0				0
	Latent period before effect	6.sec	6.Sec.	5.Sec.	5.Sec	0	4.sec.	4.sec.				6.sec
Effects on Heart Rate.	Rate per Min. before Injection	204.	186.	186.	180.	180.	180.	186.				216.
	Rate during time of action	235.	174.	186.	180.	—	178.	Lowest Point 228	234.	216.	198.	216.
	Rate after Bld. P. Returned to Normal	240.	186.	192.	174.	180.	180	190			190.	216.
Time	of Action	2 Min.	62 Sec.	49 sec.	40 sec.	0.	50 sec.	32 min.				76 Sec.
Respiratory Effects		0	0	0	0	0	0	Resp. Shallow	Slightly Slowed	Slightly Slowed	Normal	0.

Notes and Remarks.

#VIII A dose with same Protein content as the Residue, gave no effect, similarly with Iodine.
#IX Inactive in doses corresponding to Prot. and I. content of Thyroid Res. Dose inc. Ten times.
#X Iodine content of dose much smaller than that of Res.
#XI. Only a Trace of Iodine could be detected. Enormous dosage was required to give any effect on Bld. Pressure.
#XII The Protein and Iodine content of the solution, prepared from the commercial preparation was proportionately the same as the Prot. and I. content of the Dessicated Thyroid.
#XIII Tracings #1-2-3- of XIX are consecutive #4 is taken when Bld. P. is about normal. The Large dose was given to test the toxic effect of the Residue. Time between 3 and 4 = 22 min. 31 sec. in which the rise of Bld. P. was gradual and heart action regular.
#XIV This basic dose of Residue was given directly after the Large dose #XIX to check the condition of Bld. P. and Heart action.

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5. The depressor effect of the "Residue" follows only after its intravenous injection. It is not manifested after subcutaneous injection.

6. Tachycardia is not regularly produced by this depressor substance although a very large dose (10 cc.) does accelerate the pulse rate at the lowest points of blood pressure. Later the force and rate of the heart beat return to normal with the return to normal of the blood pressure. (See tracings XIX[1], XIX[2], XIX[3], XIX[4].

7. The active portion of the "Residue" is not precipitated by basic lead acetate; is not altered by boiling nor by passage through a Berkefeld filter; and is soluble in alcohol.

8. No immunity to the depressor effect of the "Residue" or of its active portion was noted during the limit of the experiments (4–6 hours).

REFERENCES

(1) Oliver and Schafer: Journal of Physiol., 1895–1896, xviii, 276.
(2) Haskovec: Wien, Med. Blät, 1896, xix, 115, 166.
(3) Georgiewsky: Zentral f. d. Med. Wissensch., 1895, xxxiii, 456.
(4) Fennyvessy: Wien. klin. Wochs., 1900, xiii, 125.
(5) Svehla: Archiv f. Exp. Path, und Pharmacal., 1900, xliii, 321.
(6) Heirratz: "Das alte und das neue über die Schildrüse." Dissertation 1894.
(7) Livon: Compt. Rend. Soc. di Biol., 1898, v, 98.
(8) Asher n. Flack: Zeits. f. Biol., 1910–1911, lv, 83.
(9) Popielski: Arch. f. die Ges. Physiol., 1911, cxxviii, 222.
(10) Lampe, Klose und Liesegang: Beit. z. Klin. Chir., 1912, lxxvii, 601.
(11) Bardenhewer: Arch. f. Klin. Chir., 1912, xcvii, 729.
(12) Black and Sanford: Journal American Medical Assoc., 1914, lxii, 117.
(13) Von Furth and Schwartz: Archiv f. d. Ges. Physiol., cxxiv, 361.
(14) S. Vincent: Internal Secretions and the Ductless Glands. E. Arnold, London, 1912.
(15) Frankel: La Semaine Medical, 1895, p. 511.
(16) Baumann: Zeits. f. Physiol. Chem., 1896, xxii, 1.
(17) Oswald: Zeits. f. Physiol. Chem., 1910, lxviii, 173.
(18) V. Cyon and Oswald: Arch. f. d. Ges. Physiol., lxxxiii, 199.
(19) Barbera: Archiv f. d. Ges. Physiol., 1900, lxix, 312.
(20) Von Cyon: Die Gefassdrusen als Regulatorische Schutzorgone des Zentralnervensystem, Berlin, Springer, 1910.
(21) Asher and Frank: Zeits. f. Biol., 1910–1911, iv, p. 83.
(22) Kraus und Friedenthal: Berl. Klin. Wochenschrift., 1908, xxxviii, 604.
(23) Riggs: Amer. Journ. Physiol., xxxiv, No. 1.

THE INFLUENCE OF OXALATES, CITRATES AND TARTRATES ON THE ISOLATED HEART

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The action of salts forming insoluble calcium compounds has frequently been explained by their physico-chemical properties. Disturbance of the calcium mechanism was, with very few exceptions, urged as the only cause underlying the changes produced by the soluble salts of oxalate, citrate and tartrate when brought in contact with the living cell. According to one view, which may be considered the dominant theory, this is accomplished by the precipitation of calcium, while another explanation assumes that the action may be due to the transformation of calcium into a non-ionized condition. The evidence on which these theories were based was derived by various methods of inquiry. Marked disturbance of calcium metabolism following the administration of soluble oxalate to animals is one of the arguments employed in favor of the contention that the mode of action of this salt consists in depriving the body of this important metal. According to Caspari,[1] who experimented on dogs, and Luitblein,[2] who employed rabbits, the elimination of calcium is greatly increased after the administration of sodium oxalate. Corroborative evidence of decalcification was furnished also by the chemical studies of Sarvonat and Roubier,[3] who analyzed the skeleton and soft parts of three guinea pigs which had been receiving sodium oxalate for 6 to 7 days. The amounts of calcium found, according to their observations, were much reduced as a result of the treatment, the soft parts being affected before the

[1] Caspari: Centralblatt f. Agricultur Chemie, 1897, **26**, p. 529.

[2] Luithlein: Archiv für experimentelle Pathologie u. Pharmakologie, 1912, **69**, p. 375.

[3] Sarvonat and Roubier: Comptes Rendus Societe de Biologie, 1911, **71**, p. 104.

bony tissues. It is interesting to note in this connection that according to Dixon's[4] observations the prolonged administration of oxalates to rabbits resulted in changes in the bones resembling rickets. The observations of Loew,[5] who reported that oxalic acid is not toxic to molds which grow without calcium, have been looked upon as especially favorable to the calcium theory.

But the main support for the contention that the calcium of the tissues is in some way concerned in the changes produced when they are acted upon by the salts under consideration was furnished by the results of the experiments indicating antagonistic action when the tissues are exposed to the combined influence of any of these compounds, and of soluble lime salts. The data which form the basis for this theory were obtained by observations under a variety of conditions and include experiments on isolated organs, as well as on the intact animal. According to the observations of Howell,[6] which were corroborated later by Locke,[7] the irritability of the gastrocnemius of the frog which is immersed in sodium oxalate may be restored by means of calcium. Januschke[8] reached the same conclusion in experiments with sodium oxalate on the isolated heart of the frog.

Recently Gates and Meltzer[9] reported experiments with sodium oxalate and magnesium sulphate in which it was shown that the action of magnesium sulphate is much more toxic when oxalate is administered at the same time. This was assumed to be due to the precipitation of calcium which, as previously shown by Meltzer and Auer,[10] antagonizes the action of magnesium. In a later communication Gates and Meltzer[11] demonstrated that the

[4] Dixon, W. E.: Manual of Pharmacology, p. 344, London, 1906.

[5] Loew: Biochemische Zeitschrift, 1912, **38**, p. 226.

[6] Howell: Journal of Physiology, 1894, **16**, p. 476.

[7] Locke: Journal of Physiology, 1894, **15**, 118.

[8] Januschke: Archiv für experimentelle Pathologie u. Pharmakologie, 1909, **61**, p. 363.

[9] Gates and Meltzer: Proceedings of the Society for Experimental Biology and Medicine, 1913, **11**, p. 23.

[10] Meltzer and Auer: American Journal of Physiology, 1908, **21**, p. 400.

[11] Gates and Meltzer: Proceedings of the Society for Experimental Biology and Medicine, 1914, **11**, p. 97.

anesthesia and paralysis produced by subminimum doses of sodium oxalate and of magnesium sulphate may be promptly abolished by the administration of calcium chloride. The same conclusions were reached by other observers who studied the action of these salts on the intact animal as well as on isolated organs. Froelich and Chiari[12] observed increased irritability of the sympathetic system by the injection of sodium oxalate. This was shown by the greater mydriatic effect of adrenalin and by a rise of blood pressure which were readily counteracted by means of calcium chloride. According to Decottignie[13] the toxicity of sodium oxalate may be decreased by the previous administration of calcium chloride or glycero-phosphate of calcium, so that even a surely fatal dose of the oxalate may be thus survived. Sugimoto's[14] experiments on the isolated uterus of the guinea pig have likewise furnished evidence of this antagonistic action. When 5 mgm. of sodium oxalate were added to 10 cc. Ringer's Solution containing 0.01 pituglandol, marked relaxation with diminished pendular movements occurred immediately. These effects were readily antagonized by adding an equivalent amount of calcium chloride.

In experiments with sodium citrate Sabbatani[15] succeeded in preventing coagulation of blood and lymph in vitro, as well as in the intact animal. The antagonistic action of calcium chloride and citrate was also demonstrated by this writer. Since calcium citrate is quite soluble he concluded, however, that its inhibiting effect on coagulation was due to the transformation of calcium into a non-ionized condition. He was led to the same conclusions by his experiments with rennin. The non-coagulability of sweet milk, he believed, may be explained by the presence of calcium in the form of the citrate. The action of sodium citrate on rennin and the antagonistic effect of calcium were corroborated

[12] Chiari and Froelich: Archiv für experimentelle Pathologie u. Pharmakologie, 1911, **64**, p. 214.

[13] Decottignie: Dissertation, Paris, 1901–1902.

[14] Sugimoto: Archiv für experimentelle Pathologie u. Pharmakologie, 1913, **74**, p. 27.

[15] Sabbatani, L.: Archives Italiennes de Biologie, 1901, **36**, p. 417.

later by Vietinghoff-Scheel.[16] Loeb,[17] experimenting with striated muscle, observed that it contracts powerfully or it may even go into tetanus when brought in contact with air, oil, or other indifferent solutions after previous immersion into solutions of sodium tartrate, oxalate or citrate. Since this phenomenon, which he termed contact irritability, may be inhibited by adding small amounts of calcium chloride to substances that are calcium precipitants, Loeb concluded that decrease of the calcium ions in the tissues is the cause of this peculiar action. He made, however, the plausible statement that these salts may have a specific effect upon other constituents of the protoplasm, such as protein. The calcium theory was also suggested in explanation of the action of the purgative salts. Loeb observed that salts which precipitate calcium are the most powerful stimuli of nerve and muscle and that they also exert a purgative effect. Their action would, therefore, be the same whatever the mode of administration, for contact with the cell is all that is necessary in order to produce the desired effect. Macallum[18, 19] and Bancroft[20] put this theory to an experimental test. Experiments with citrates and tartrates given by subcutaneous injection into rabbits produced purgation which could be inhibited by calcium chloride. Their results would seem to be contradicted, however, by the experiments of Auer,[21] as he failed to produce purgation in rabbits by the subcutaneous injection of sodium citrate. That the action of this salt is due to the formation of a compound with calcium was contended later by Robertson and Burnett.[22] According to these observers tolerance to sodium citrate may be produced in rabbits and is due to adaptation to a lower calcium level.

The calcium theory, it may be noticed, has gained adherents among many workers who sought an explanation for the pharma-

[16] Vietinghoff-Scheel: Archives internationales de pharmacodynamie et de therapie, 1902, **10**, p. 145.

[17] Loeb: American Journal of Physiology, 1901, **5**, p. 362.

[18] Macallum: University of California Publications, Physiology I, 1903, p. 5.

[19] Macallum: University of California Publications. Physiology II, 1905, p. 65.

[20] Bancroft: Journal of Biological Chemistry, 1907, **3**, p. 191.

[21] Auer, J.: American Journal of Physiology, 1906, **17**, p. 15.

[22] Robertson and Burnett: Journal of Pharmacology and Experimental Therapeutics, 1912, **3**, p. 635.

cological action of oxalates, citrates and tartrates. A notable exception to this view was taken, however, by Gros[23] in a recent communication on this subject. He found in experiments on the isolated frog heart that sodium citrate is decidedly more toxic than sodium oxalate, which is a fact of considerable importance for calcium citrate is quite soluble, a saturated solution at room temperature being about 1 gram per liter, while calcium oxalate is practically insoluble. The calcium precipitation theory is, therefore, untenable according to this observer.

In the course of investigations on the action of sodium tartrate on the circulation carried on in this laboratory it was observed that the effect of this salt may be promptly abolished by the intravenous injection of sufficient quantities of calcium chloride. The antagonistic action of the calcium and tartrate in the intact animal pointed therefore to disturbance of the calcium mechanism as the cause of the action of the tartrate ion. It seemed, however, that a more definite answer concerning this mechanism might be obtained by making a comparative study of the action of salts closely allied with respect to the solubility of their calcium compounds. This coupled with the obvious advantage to the solution of a problem gained by experimentation under conditions much less complicated led to the systematic study of sodium tartrate, citrate, and oxalate on the isolated heart.

METHODS

The isolated heart of the dog, cat and frog was used in these experiments. Perfusion of the mammalian heart was carried out as follows: The animal was anesthetized and bled from the carotid artery, Locke's solution being introduced at the same time into the femoral vein. We found that the amount of blood removed was an important factor in the success of the experiment. The heart of the animal that was bled until it was almost exsanguinated frequently failed to give satisfactory results. It soon became oedematous and after a short time it stopped in systole. Uniformly good results were obtained, however, when bleeding

[23] Gros: Archiv für experimentelle Pathologie u. Pharmakologie, 1913, 71, p. 395.

was moderate or, better still, when the subject was not bled at all before removal of the heart. The Langendorff apparatus was used, a double walled glass chamber in which the heart was suspended being substituted for the original metallic chamber as recommended by the author of this method. It was raised above the bath and kept at the same temperature as the perfusing fluids by circulating water in the intervening space.

The pressure in the perfusion fluids and in the heart was carefully regulated, all experiments being carried out under uniform pressure. The same precaution was observed with regard to the temperature of the fluid as it entered the heart. Special tests, however, to ascertain the effect of temperature have shown that small differences do not materially influence heart action. Changes in pressure, on the contrary, have produced considerable differences in cardiac action.

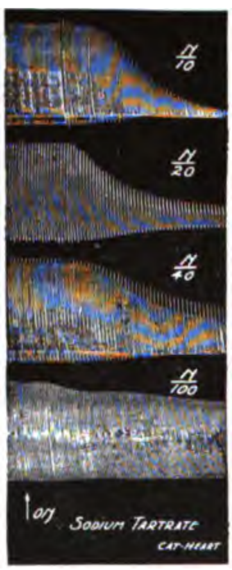
The frog's heart was perfused by means of a cannula which was introduced into the sinus venosus, the other veins being tied. The fluid thus passed through the heart, leaving it by way of the aorta. The solutions used for perfusion were contained in reservoirs which terminated at the lower end into glass tubes provided with stop cocks. By means of rubber tubing each reservoir was connected with the heart cannula. A free flow of the solutions was allowed, the perfusing fluids being thus replaced continually. The fluids in the reservoirs were kept at approximately constant pressure.

Experiments with Sodium Tartrate

Previous investigations on this subject have been made by Karczag,[24] who observed decreased cardiac activity and heart block when the isolated heart of the turtle was perfused with $\frac{N}{100}$ tartaric acid. Depressed heart action was also obtained by Gros[25] and by Sakai[26] in experiments with tartrates when the isolated heart of the frog was perfused.

[24] Karczag: Zeitschrift für Biologie, 1909, **53**, 218.
[25] Gros: l.c.
[26] Sakai: Zeitschrift für Biologie, 1914, **64**, p. 1.

Our own investigations with sodium tartrate, which were carried out on the heart of the dog, cat and frog, have likewise shown depressed heart action which varied, with the concentration of the solution used. Thus perfusion of the cat's heart with solutions of $\frac{N}{10}$, $\frac{N}{20}$, $\frac{N}{40}$ and $\frac{N}{100}$ sodium tartrate in defibrinated blood, diluted with Locke solution, or in Locke solution alone, was followed by diminished activity of the heart which became more marked as the concentration of sodium tartrate was in-



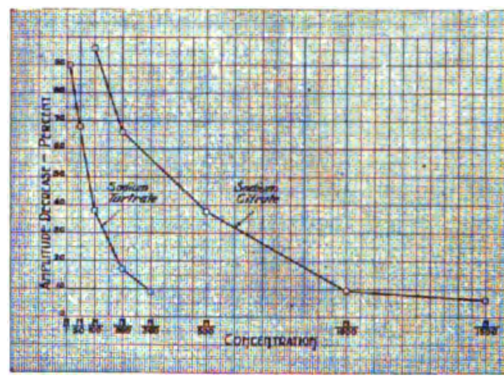


Fig. 2. Graphic comparison of effect on the frog-heart of equivalent quantities of sodium citrate and sodium tartrate dissolved in Ringer solution. Perfusion time in all cases 30 seconds, each point representing an average of at least two determinations. The difference between the smallest amplitude obtained on perfusion with a given concentration, and the amplitude just before perfusion, divided by the latter quantity gives the amplitude decrease in per cent.

Fig. 1. The response of the isolated cat-heart to four concentrations of sodium tartrate in Locke solution.

creased (fig. 1). It may be remarked, however, that the action did not vary in the same ratio as the concentration. The effect of various dilutions was even better exemplified in experiments on the frog's heart (fig. 3) in which very dilute solutions were employed. When $\frac{N}{800}$ sodium tartrate was perfused for 30 to 60 seconds a slight cardiac depression was observed, the systole

alone being decreased in some experiments, in others the decrease affected diastole as well as the systole. This may be regarded as the minimum concentration which can produce any effect since a $\frac{N}{400}$ solution proved to be without any action. Cardiac activity was markedly depressed under the influence of $\frac{N}{200}$ sodium tartrate, the effect on the systole being more pronounced than on the diastole. This was also observed with more concentrated solutions. Although the effects varied with the concen-

Fig. 4. Shows the effect of perfusion time on the toxicity of sodium citrate.

Fig. 3. Tracings showing the results of 30-second perfusions with various concentrations of sodium tartrate in Ringer solution. When the heart is not working very strongly only systole is affected ($\frac{N}{10}$) while both systole and diastole are affected when the contractions have a large amplitude. Compare this figure with figure 6 showing action of sodium citrate.

tration of the salt, their ratios, as was observed in experiments with the cat's heart, were likewise unequal. This is apparent on comparing the action of $\frac{N}{100}$, $\frac{N}{50}$ and $\frac{N}{10}$ solutions of sodium tartrate, the decrease in amplitude being respectively 40, 70 and 90 per cent (figs. 2 and 3).

A very noticeable difference in the action of sodium tartrate was also obtained by varying the perfusion time. $\frac{N}{10}$ sodium tartrate perfused for one minute produced a marked decrease in amplitude in the dog's heart. When the perfusion time was increased to $1\frac{3}{4}$ or more minutes, cardiac action was much weaker and also became irregular. The effect of time was also noticeable in experiments with shorter intervals, and as will be shown later, was especially striking in experiments with sodium citrate (fig. 4).

Experiments with Sodium Citrate

There is a noteworthy paucity in the literature, of studies on the action of citrates on the circulation. Vietinghoff-Scheel[27] reported some experiments on the effect on blood pressure while a short notice was recently published by Gros,[28] Clark[29] and later by Sakai[30] on its influence on the isolated frog heart. We are not aware of any other published communications on the subject. In the experiment to be reported sodium citrate, dissolved in Locke solution, was employed in various concentrations, the cat heart and that of the frog being used. The effect, a description of which follows, may be considered typical of the action of sodium citrate on the heart of the cat.

A solution of $\frac{N}{10}$ sodium citrate produced complete cessation of cardiac action, which occurred about 30 to 40 seconds after the solution was turned on (fig. 5). For two minutes after the citrate was discontinued, Locke solution being substituted in its place, the heart was at a standstill. Although considerable improvement took place, the recovery was incomplete and very slow. Eight minutes after perfusion the contractions were still weak, the amplitude being about one-sixth the size before perfusion with the citrate. It may be remarked, however, that the rhythm was regular at this time.

[27] Vietinghoff-Scheel: l.c.
[28] Gros: l.c.
[29] Clark: Journal of Physiology, 1913, **47**, p. 66.
[30] Sakai: l.c.

The effect of concentration was well shown in experiments with weaker solutions. $\frac{N}{20}$ sodium citrate (fig. 5) perfused for one minute was just sufficient to suspend heart action about the end of the period, but the contractions were just as vigorous one minute after Locke solution was turned on as before the citrate was perfused, the amplitude continuing to increase during the next 30 seconds until it became 30 per cent greater than it was during the fore period. The rate was only slightly accelerated. A number of experiments with $\frac{N}{40}$ solution was also carried out. In every case heart action was reduced considerably, the individual beats becoming very feeble in some instances, in others they were still fairly strong at the end of one minute perfusion with citrate. Heart action, however, was never inhibited

Fig. 5. Action of two concentrations of sodium citrate in Locke on the cat's heart. Cf. figure 1.

completely as was observed with stronger solutions. Except in one experiment the activity of the heart in the after period was greatly augmented, the force being appreciably greater on recovery, but, as was already stated under sodium tartrate, this lasted a brief period after which it became normal again. It is worthy of notice that rhythm was not appreciably affected during these experiments.

Experiments with citrate on the isolated frog heart have shown even more clearly the influence of concentration (figs. 2 and 6). It was found that a very dilute solution, $\frac{N}{1500}$, sodium citrate perfused for two minutes was still active though very slightly so but a solution of $\frac{N}{1000}$ perfused for 30 seconds to one minute showed a well marked decrease in amplitude with prompt

recovery when the perfusion with the citrate was discontinued and Ringer or Locke solution allowed to pass through the heart. Solutions of $\frac{N}{500}$, $\frac{N}{400}$, $\frac{N}{200}$ and $\frac{N}{100}$ were also tested. As shown in figure 6 the force of cardiac contraction diminished as the concentration was increased. The amplitude after perfusion with a $\frac{N}{100}$ solution for 30 seconds promptly diminished so that the heart beats were noticeable when magnified. Attention may be called here to the interesting phenomenon already observed

Fig. 6. Tracings of the effect of sodium citrate in Ringer on the frog-heart. Cf. the extent of the effects observed here with that in figure 3.

with sodium tartrate, namely, the augmentation of cardiac action in the after period. This was even more marked with sodium citrate, especially when concentrations above $\frac{N}{1000}$ were employed. In some experiments the amplitude was doubled when Ringer or Locke solution was perfused after previous treatment with citrate but the rhythm was not disturbed. There was never any irregularity such as was observed under similar

conditions in the cat's heart. The decrease in amplitude with different concentrations is best shown in figures 2 and 6. It will be noticed that the decrease is not proportional to the increase in concentration. Thus after perfusing with $\frac{N}{500}$, for example, the amplitude decreased 38 per cent; when the concentration was $\frac{N}{200}$ the decrease was about 65 per cent.

Attention may be called in this connection to the influence of the perfusion time. As is shown in figure 4 marked differences were observed. When perfused for 10 seconds depression was pronounced but the contractions were good at the end of the experiment, recovery being prompt. But when the same heart was perfused for 30 seconds, cardiac depression was greater and recovery was delayed.

The Comparative Action of Tartrate, Citrate, and Oxalate

If sodium tartrate and citrate be now compared it will be observed that in the same concentration the citrate is a more powerful depressant than the tartrate. This is shown with great clearness in experiments on the frog heart. Thus the amplitude, as shown in figures 2, 3 and 5, was decreased nearly 10 per cent with $\frac{N}{800}$ sodium tartrate, while the decrease with $\frac{N}{800}$ sodium citrate was 50 per cent. It may be remarked here that $\frac{N}{400}$ sodium tartrate had no effect on the frog heart while this concentration of sodium citrate decreased the amplitude approximately 45 per cent. The difference was about the same with $\frac{N}{200}$ solutions, while a solution of $\frac{N}{100}$ showed a decrease of 38 per cent with sodium tartrate and 95 per cent with sodium citrate. The results obtained with the heart of the dog and cat have similarly shown that sodium tartrate is much weaker than citrate. This is well illustrated in figures 1 and 5 in which the effects of $\frac{N}{10}$ and $\frac{N}{20}$ of the tartrate and citrate are shown. Similar results were obtained with $\frac{N}{40}$ solutions.

The comparative action of sodium citrate and sodium oxalate was also studied. Owing to the insolubility of calcium oxalate in neutral salt solutions a Locke solution, minus calcium, was used in which the salts to be tested were dissolved. An isotonic sodium chloride solution was also tried, but this was abandoned

as it was found unsatisfactory. Heart action became feeble and uncertain when perfused with this solution. Gros,[31] and more recently Arima,[32] reported that a sodium chloride solution alone may sustain the heart of the frog. Their method, however, was quite different from the one employed in the present investigation. They filled the heart with small quantities of fluid, 1 to $1\frac{1}{2}$ cc., which they allowed to remain for some time instead of continually changing the perfusing fluid as was done in our experiments. According to Arima the immediate effect of such a solu-

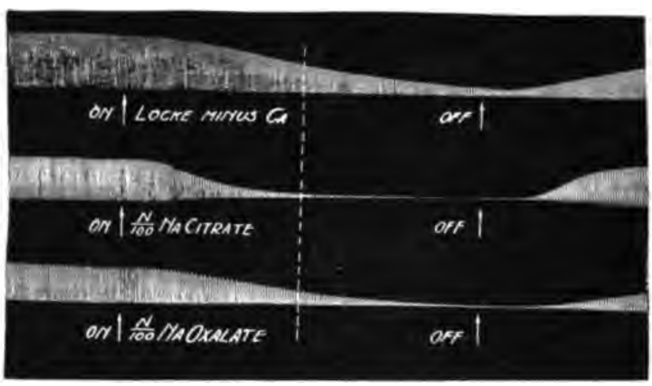


Fig. 7. Effect on the isolated cat-heart of sodium citrate and sodium oxalate dissolved in Locke solution minus calcium. The effect of a Locke no calcium solution on the same heart is shown for comparison. The perfusion time is two minutes, the broken line marking the close of one minute's perfusion. Citrate is seen to be the more toxic anion.

tion, even when small quantities were used, was to bring the heart to a standstill, from which it recovered, however, within ten to fifteen minutes.

Control experiments with calcium-free Ringer or Locke solution, which we carried out on the hearts of cats and frogs, indicated a decided decrease in cardiac activity, but the effect was much greater when such a solution containing either citrate or oxalate was perfused for the same length of time (see figs. 7, 8 and

[31] Gros: l.c.
[32] Arima: Archiv für die gesammte Physiologie des Menschen und der Thiere, 1914, **157**, p. 531.

9). In some experiments a moderate activity of the heart was still present at the end of two minutes' perfusion with a calcium-free solution, which was not the case when perfused with such a solution containing even moderate amounts of citrate or oxalate.

Comparative tests with citrate and oxalate were carried out with equi-molecular solutions of these salts, dissolved in Ringer

Fig. 8. Effect on the frog-heart of alternate perfusion with sodium citrate and sodium oxalate dissolved in Ringer minus calcium. Perfusion time is 10 seconds, there being two minutes between perfusions.

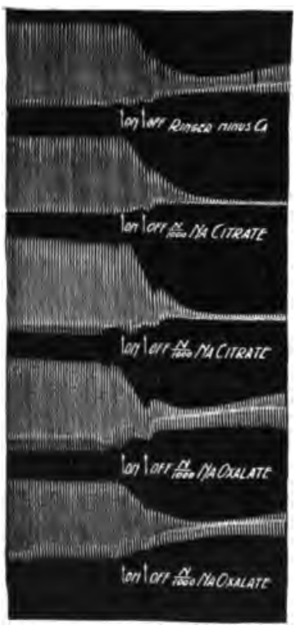


Fig. 9. Two consecutive perfusions with citrate, and two with oxalate give results on another frog-heart, again denonstrating the greater toxicity of citrate. The rate of perfusion in this heart was greater than that in figure 8.

or Locke—minus calcium. The results obtained indicate that in some cases oxalate has the same activity as citrate, but in a much larger number of experiments the citrate was decidedly stronger than the oxalate. This is particularly well illustrated in experiments on frogs when the perfusion time lasted only ten seconds

(figs. 8 and 9). When the perfusion time was longer the difference was much less marked. In experiments on the cat's heart similar results were obtained (fig. 7). A solution of $\frac{N}{100}$ (fig. 7) sodium citrate at the end of one minute's perfusion caused a considerable decrease in heart action, the amplitude as shown in the figure being markedly diminished. When $\frac{N}{100}$ sodium oxalate solution was perfused through the same heart four minutes later, the diminution of heart action at the end of one minute's perfusion was noticed to be much less marked. Recovery, however, was somewhat slower than after the citrate. A more concentrated solution has also shown that the citrate is more active than the oxalate. This appears very strikingly with perfusion for one minute of $\frac{N}{40}$ sodium citrate and $\frac{N}{40}$ sodium oxalate. Complete cessation of cardiac action for more than thirty seconds followed perfusion with citrate, while perfusion with oxalate of the same concentration seven minutes later caused a very marked decrease in amplitude, but the heart was contracting, though feebly, at the end of the period. Recovery in this case, too, was rather slow. The heart failed to attain the same strength as after the citrate.

DISCUSSION

As observed in the introductory remark, precipitation of calcium in the cell and tissues was offered as an explanation of the action of tartrate, oxalate, and citrate. The results we obtained we believe justify the conclusion that this theory is untenable, for if the precipitation of calcium in the tissues were the cause it would follow that the salts which form the most insoluble compound with this metal, would be the most toxic. According to this theory sodium oxalate ought to be the most toxic of the three salts under consideration, for its calcium salt as given by Richards, McCaffrey and Bisbee[33] is very slightly soluble in neutral solution, while sodium citrate ought to be the least active because calcium citrate is the most soluble of the three salts. Its solubility was found to be 0.0959 grams per 100 cc. according

[33] Richards, McCaffrey and Bisbee: Zeitschrift für anorganische Chemie, 1901, **28**, p. 85.

to Partheil and Hübner.[34] Sodium citrate ought, therefore, to be also less toxic than sodium tartrate for the solubility of calcium tartrate, as determined by Cantoni and Zachoder,[35] is 0.0525 per 100 cc., which is about half that of calcium citrate. Our experiments have unmistakably shown, however, that sodium citrate is the most toxic, while sodium tartrate is least toxic. The comparative strength of these three salts is illustrated in figures 2, 3, 8 and 9.

Transformation into non-ionized compounds was the other theory suggested to explain the action of these salts. Experiments undertaken to test this theory utterly failed to furnish any evidence of such a change. When the heart of the cat was perfused with solutions of calcium tartrate or citrate in various

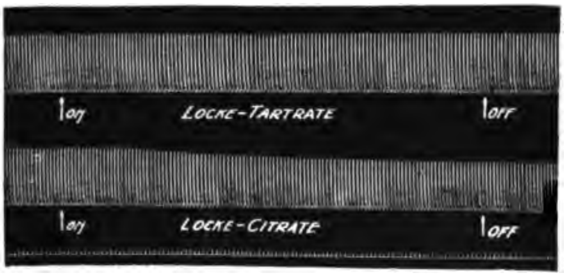


Fig. 10. Locke-tartrate means a Locke solution in which the $CaCl_2$ was replaced by an equivalent quantity of Ca tartrate. The toxic effect of the small quantity of tartrate ion is completely masked by the effect of the Ca ion. Locke-citrate is a Locke solution in which $CaCl_2$ is replaced by an equivalent of Ca citrate. The greater toxic action of the citrate ion is shown by the inability of the Ca ion to completely mask its effect. The record was taken with the isolated cat-heart.

concentrations it was found that these salts could be as readily utilized as calcium chloride. Figures 10 and 11 show that when experiments were conducted with Ringer or Locke solution, calcium citrate or tartrate being used instead of calcium chloride, the same amount of calcium being contained in the solution in each case, the results obtained were the same. Little or no difference due to the tartrate or citrate ion could be observed.

[34] Partheil and Hübner: Archiv der Pharmazie, 1903, **241**, p. 413.
[35] Cantoni and Zachoder: Bulletin de la societé chimique de France, 1905, **33**, p. 747.

In this connection Clark's[36] observations are exceedingly interesting. He found that the insoluble calcium oleate formed by adding its soluble sodium salt to Ringer's solution can revive the isolated frog heart that has been fatigued by continuous perfusion with this fluid.

Attention may also be directed to the rapid action of the salts observed in our experiments in which perfusion for ten seconds only was sufficient to produce marked effects. No satisfactory explanation can be offered at present, but in the light of the investigations on the permeability of the cell to organic

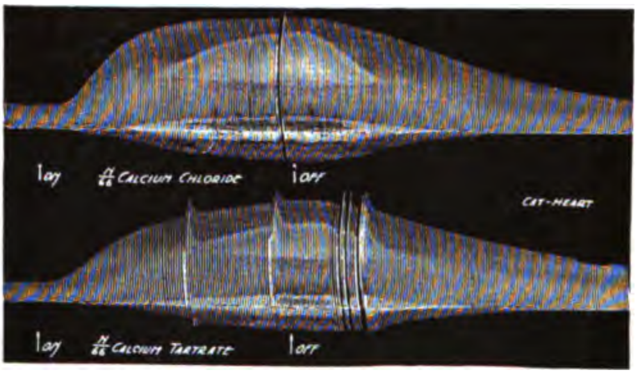


Fig. 11. Effect on the cat's heart of equivalent quantities of $CaCl_2$ and Ca tartrate dissolved in Locke. This, as well as figure 10, shows that the toxicity of tartrate and citrate cannot be attributed to a transformation of calcium into compounds that the heart cannot "use."

acids and neutral salts, surface action suggests itself. According to recent observations of Harvey[37] the rate of diffusion of oxalic, tartaric and citric acid into the cell is twelve, fifteen, forty and thirty minutes, respectively, while Overton[38] previously demonstrated that organic salts penetrate into the cell even much more slowly than their acid. Harvey[39] has also shown that while the cell is permeable to some dyes in the acid condi-

[36] Clark: l.c.
[37] Harvey: Science, N. S., 1914, **39**, p. 947.
[38] Overton: Archiv für die gesammte Physiologie, 1902, **92**, p. 115.
[39] Harvey: J. Experimental Zoology, 1911, **10**, p. 507.

tion, it resists their extrance as a neutral salt. It has been suggested that the action of kations is also extracellular. Overton[40] claimed that potassium and calcium penetrate the striated muscle fiber very slightly if at all and maintained, therefore, that the action of electrolytes on striated muscle is confined to the surface. Boehm[41] more recently adopted the same explanation for the action of potassium and calcium on the isolated heart of the frog, for the rapid action of their salts would have been impossible if the effect observed depended upon their penetration into the muscle cell. The prompt recovery observed in our experiments when Ringer and Locke solution was perfused after the previous treatment with citrate, tartrate, or oxalate may also be regarded as additional evidence against internal changes produced by these salts. If any damage were done to the cell, as might indeed be expected if these substances penetrated into the interior of the muscle fiber, it is extremely doubtful whether the heart could resume its normal activity almost momentarily and not only make a perfect recovery but even exhibit for an appreciable length of time augmented activity without any untoward after effects. No definite statement can be made, however, as to the nature of the extracellular changes involved. Whether it is due to loss of lipoids, as suggested by Clark's[42] observations, or to some physico-chemical changes at the surface of the cell cannot be told at present for our knowledge of the factors concerned in the phenomena observed is as yet wholly inadequate.

SUMMARY AND CONCLUSIONS

1. The results obtained in this investigation show that sodium tartrate is much less active than citrate or oxalate.

2. Equi-molecular solutions of the latter perfused for the same length of time indicate that sodium citrate may be more toxic than oxalate.

[40] Overton: Archiv für die gesammte Physiologie, 1904, **105**, p. 176.

[41] Boehm: Archiv für experimentelle Pathologie und Pharmakologie, 1914, **75**, p. 230.

[42] **Clark:** l.c.

3. The action varies with the perfusion time, the longer the heart was perfused the greater the toxic effect.

4. The data obtained do not support the calcium precipitation theory as an explanation of the action of these salts since the effect seems to be independent of the solubility of their compounds with calcium. Our conclusions are therefore in harmony with those of Gros.

5. The theory that the action is due to the formation of non-ionized calcium salts is likewise untenable for calcium tartrate and citrate can be utilized by the heart.

6. The rapid action indicates that the changes produced are not due to penetration into the cell since, as was found by Overton and Harvey, diffusion of neutral salts into the cell takes place very slowly.

It is suggested, therefore, that the effects are due to extracellular changes.

AUTOLYSIS AND INVOLUTION

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Our knowledge of *in vivo* autolysis rests upon comparatively few observations. Martin Jacoby[1] found free leucin and tyrosin in necrotic portions of ligated liver.

Green[2] on the botanical side, described autolytic changes in seed germination; other botanists[3] have likewise done this although Pond[4] failed to find the date-seed embryo capable of self-digestion. P. A. Levine[5] found evidences of autolysis in developing eggs of fish and fowl. The circumstantial evidence that the only adequate explanation of disuse atrophy and kindred phenomena is autolysis may complete our invoice of data upon autolysis in the body.

Three years ago, the present writer conceived the idea of studying the process of involution in the larval frog with special reference to the possibility of autolysis being the principal factor, for here is an ever-available supply of material. With the newer methods for studying small amounts of material (Folin's, Van Slyke's, Abderhalden's, Sörensen's, etc.), the problem would not seem difficult. In reviewing the literature, which is quite extensive,[6] it became evident that no work had been performed upon the physiology of the problem, all having been concerned

[1] Zeitschr. f. physiol. Chemie, 30, p. 149.

[2] Proc. Roy. Soc. London, 47, p. 147.

[3] Grüss: Ber. D. bot. Ges., 11, p. 288; 12, p. 60. Brown and Morris: Journ. Chem. Soc., 57, p. 458. Van Tieghem: Comptes rendus, 84, p. 582. Gris: Ann. Sci. Nat. Bot. (5) 2, p. 90. Hansteen: Flora, 79, p. 419. Linz: Jahrb. wiss. Bot., 29, p. 267.

[4] Annals of Botany, January, 1906.

[5] Zeitschr. f. physiol. Chemie, 35.

[6] Cf. Mercier: Arch. d. zool. expér. et gén. (4) 5, p. 1, for bibliography.

with histological aspects. Phagocytosis was accepted almost in every case as the efficient factor, dating from the classic investigations of Metschnikoff[7] on the absorbing tail of the tadpole of the frog, which became the foundation for his theory of phagocytosis. One, alone amongst the number of students of involution advocated any process other than phagocytosis (Looss).[8] While Looss had nothing other than histological data to present, yet he advocated the process of physiological absorption—"einer Auflösung, mit einer Resorption im strengen Sinne des Wortes."[9] The tail he likens to "der Dottersack für den sich entwickelnden Embryo" and like the yolk-sac, "die Dottermaterial wird ohne Hülfe von Leuko- oder Phagocyten aufgebraucht." For insects, during the period of metamorphosis, the exhaustive researches of Janet[10] show that "au cours de la dégénérescence, c'est-ā-dire depuis le moment ou le muscle est encore intact jusqu'à celui où il n'en reste plus d'autre trace que les enveloppes de ses faisceaux, il n'y a certainement aucune phagocytose c'est-à-dire aucun englobement, par un leucocyte amiboïde, de particules solides du muscle en histolyse."[11] The process is conceived as "une histolyse par digestion cavitaire due à l'action des diastases du sang lesquelles diastases, sans action sur le tissu vivant, apte à se défendre l'attaquent des qu'il est mort."[12] In this opinion, Janet is supported by Korotneff,[13] Miall and Hammon,[14] Karawaiew,[15] Rengel,[16] Berlese,[17] Terre,[18] et al.

[7] Biologisches Centralb., 3, p. 560.

[8] Looss: Preisschriften von der Fürstlich Jablonowski'schen Gesellschaft in Leipsig. XXVII. Verlag von B. S. Hirsel in Leipsig.

[9] L. c., p. 91.

[10] Anatomie du corselet, etc. Limoges: Ducourtieux et Gout (privately printed).

[11] P. 134.

[12] P. 133.

[13] Biol. Centralb., 12, p. 261.

[14] Trans. Linnean Soc. (2) 5, p. 265.

[15] Biol. Centralb., 19, p. 122.

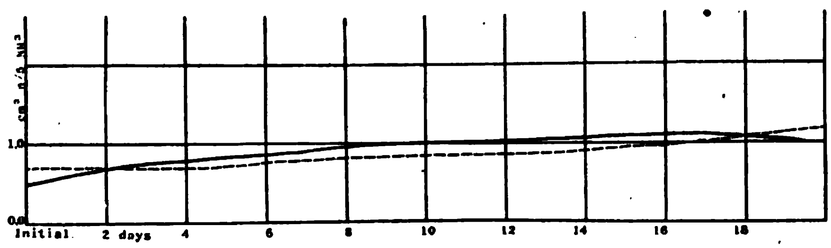
[16] Zeitschr. f. wiss. Zool., 62, p. 1.

[17] Zool. Anzeiger, 23, p. 441.

[18] Bull. Soc. entom. France, 1900, p. 62.

Atrophy, such as occurs in muscle degeneration in man (myositis, dermatomyositis, etc.) is nearly universally ascribed to a process involving phagocytes.[19] How much the atrophy of disuse is interpretable upon this theory, or upon any other is unknown at present. The similarity of histological pictures and descriptions of muscle degeneration and atrophy in the larval frog's tail is striking, lending a certain basis to the belief that fundamentally, there is a correspondingly similar physiological substratum; that is, that the pathological states described by Senator, Strümpel and others are reflected in the normal absorption of the tadpole's tail.

That phagocytosis is the efficient factor in the involution of the tail of the frog larva is improbable from the following considera-



Showing the relation between autolyzing muscle in a normal condition and when it has undergone partial degeneration after its innervation has been cut. Continuous line = control; broken line = experiment.

tions: In the first place, it is exceedingly doubtful that phagocytes affect normal tissue, so that we must logically assume a precedent lesion, something perhaps as Bataillon[20] has done in regard to the growth of the urostyle, which cuts off the blood supply, leading to an asphixiation of the tail tissues. Secondly, the rapidity of the process is scarcely coördinate with the activity of phagocytes; normally, over a half of a gram,[21] dry weight, of tissue becomes absorbed during two weeks, but while this is not especially striking, what of the anomaly when on feeding thyroid,

[19] Cf. Steiner: Journ. Exp. Med., vol. 6, 1904.

[20] Comptes Rendus (9), 2, p. 137.

[21] In Rana pipiens; in Rana catesbiana, this may be tripled.

which Gudernatsch[22] has shown to accelerate the process of absorption over two-thirds, so that even three days show the complete absorption of the tail tissues.[23] Again, histological studies reveal the relatively small number of phagocytes; that the process of atrophy begins at least in some tissues (epidermis, Looss), before the advent of phagocytes; that along with the phagocytes, erythrocytes appear which are to be accounted for in these locations only by rupture of the blood vessels since diapedesis does not occur in the red cells; this rupturing seems to indicate the previously weakened condition of the walls of the vessels and of the sarcolemma (Barfurth, Looss). In myositis, the degeneration of the muscle takes place centrifugally, not centripetally, so that the core of the muscle fibres and not the periphery, where the phagocytes occur, exhibits the degenerative changes first (Steiner). Thirdly, I have shown[24] that there is no concomitant rise in leucocytes and especially of the polynuclear variety, during the process of involution, a rise which should be expected if the process is due to the activities of phagocytes derived from the blood;[25] leucocytosis should appear here as in corresponding cases in mammals.

The various theories as to the nature of the impulse causing involution which have been offered, are inadequate. Barfurth[26] suggested disuse degeneration, but anyone familiar with tadpoles will bear witness to the fact that the tail is used during swimming movements even during metamorphosis. Bataillon's[27] "asphixiation theory," involving a cutting off of the blood supply

[22] Arch. f. Entwicklungsm., 35, 457 (1912).

[23] As to what the special factor in thyroid is, the present writer has shown (Journ. Biological Chemistry, 19, No. 3, 1914) that iodin in some association with thyroid globulin or even iodized amino-acids (3, 5, diodo-tyrosin) produce the same result as intact gland substance as used first by Gudernatsch.

[24] Proc. Soc. Exp. Biol. and Med., 10, p. 31.

[25] Although Metchnikoff first postulated that the phagocytes were not blood cells, but arose *in situ*, yet the researches of Mercier have shown that the cells which Metchnikoff described as phagocytes circulate in the blood stream and in histological sections, they are shown to be leucocytes.

[26] Also: Bohn, G.: Comptes rendus, 56, p. 661.

[27] Comptes rendus (9) 2, p. 137.

is not favored by my experiments in ligating the dorsal aorta of normal and of involuting larvae with the result that no effect upon metamorphosis in any case was visible. Barfurth's[28] further idea that hunger may be the "förderndes Prinzip" can scarcely be true, for Bábak showed in 1906 that larvae do feed and moreover, the studies of Gudernatsch, myself and others, where food is administered (thyroid) show that involution occurs when food is present. The corresponding case of the Rhine salmon described by Miescher,[29] where no food is taken during the atrophy of the muscles and the formation of gonads, is not a parallel one to the larval frog, nor is the instance of larval insects the same, for in both cases, masses of food are laid up previous to metamorphosis.[30] Loeb[31] has shown that the nervous system has no influence on metamorphosis and I can corroborate this observation by my experiments upon severing the nerve cord of normal and involuting individuals.

With regard to autolysis, it must be admitted that the case at present is not clear. I have shown in another place[32] that there is no quantitatively demonstrable difference in amino-acid content in normal and in involuting larval tails. The suggestion was there made that perhaps the case was similar to that of the amino-acid content of the blood, earlier investigations failing to reveal the presence of these compounds, while later by aid of the micro methods to which I have referred at the beginning of the present article, these Bausteine were recognized by Van Slyke, Folin and Abderhalden and quantitatively estimated, even although they were rapidly withdrawn from the blood stream. But in the present instance, exactly these methods have been used.

The purpose of the following pages is to present evidence that

[28] Arch. f. mikr. Anat., 29, p. 28.

[29] Histochemisches und physiologisches Arbeiten.

[30] Storage of food does take place, of course, in the frog, to a slight extent as in the fat body, so that the case is even stronger against Barfurth's idea as to inanition.

[31] Archiv f. Entwicklungsm., 4, p. 502.

[32] Proc. Soc. Exp. Biol. and Med., 12.

in vitro autolysis is not accelerated in absorbing tissue over non-absorbing (which will be designated the control), as might be postulated if autolysis were the efficient factor.

The method used was the aseptic one of Salkowski,[33] toluene and chloroform being used for inhibiting bacterial growth. The material was kept in a thermostat at 30° C; while this temperature may not be optimum for poikilothermous animals like frog larvae, yet inasmuch as autolysis proceeded in the controls, this degree of heat does not inhibit the enzyme action.

Experiment, June 22. Tails from about twenty metamorphosing frog-larvae (*Rana pipiens*) were cut from the body at their bases, dried upon filter paper from adhering moisture and blood, balanced against a similar set from non-involuting larvae, ground in sand, made up to 250 cm.^{3} distilled water, covered with toluol and placed in a thermostat at 37° C. Left five days, shaken daily. Kjeldahl determinations upon 25 cm.^{3} aliquots of the tannic acid filtrates gave, for 25 cm.^{3} portions:—

Control	Absorbing
1.0 cm.^{3} $\frac{N}{5}$ NH^3	1.0 cm.^{3} $\frac{N}{5}$ NH^3 [34]

Similar results were obtained from a ten day period sample, showing that *no acceleration of autolysis appears in the absorbing tissue over the control.*

The above experiment has been repeated upon larvae of *Rana catesbiana*, in Connecticut, with the same results.

Experiment, August 11. 0.40 gram wet weight of material from the tails from each of a set of normal and involuting individuals of *Rana pipiens* was placed in 50 cm.^{3} Erlenmeyer flasks with 3 cm.^{3} H_2O (making thus approximately 5 cm.^{3} cultures). 2.5 cm.^{3} aliquots were examined after twenty-four hours by Van Slyke's gasometric method for amino-acids, as follows:—

	Control		Absorbing
Initial	0.350	cm.^{3} nitrogen over H_2O	0.325
24 hr. aliquot	0.385	cm.^{3} nitrogen over H_2O	0.410

These figures are all within the error of observation in the present instance, showing, again, that *no acceleration of autolysis is evident in*

[33] Die Deutsche Klinik, 11, 1903.
[34] Calculations are left thus since only relative weights were used.

absorbing over control material.[35] Taken in connection with the former experiment, I think that the case warrants the generalization that there is no evidence for acceleration of autolysis in the larval frog's tail.

It is difficult to determine the nitrogen partition of excreta, which might afford some evidence in the present case. Owing to the fact that during metamorphosis, the intestine voids itself of faeces, which contain bacterial remains, along with portions of undigested plant material, bearing nitrogen, collection of the urine is attended with unsurmountable difficulties; collection of the urine itself, if possible, involves loss of nitrogen in the form of ammonia and urea would be decomposed also. However, I placed five individuals from each of the two divisions, viz., control and absorbing, in a larger beaker containing 100 cm.^{3} distilled water and after twenty-four hours made Kjeldahls on the filtered liquid:

(100 cm.^{3} aliquot)

Control	Absorbing
0.80 cm.^{3} $\frac{N}{5}$ HCl................	1.10

Ninhydrin being negative after filtering, there is little reason to believe that this difference concerns amino-acids. Moreover, urea determinations show that it is not urea nitrogen. In all probability, the result concerns defecated matter. During the time of this experiment, the weight changes were:—Beginning, 3.04 g. twenty-four hours, 2.44 g., average of five individuals; difference, 0.60 g. = loss in weight for twenty-four hours.

That the creatin nor creatinin component is altered, I have shown in another place[36] and while this criterion of muscle atrophy is held in question by some[37] as a valid index of endogenous metabolism, yet the point is of interest here.

It is impossible, therefore, with our present knowledge, to state whether involution in the larval frog involves a closed system, that is, that the materials from the tail act as "Dotter-material" for the developing embryo as Looss suggested, and as

[35] The difference of 0.019 mmg. amino-nitrogen of the control and of 0.046 mmg. in the case of the absorbing tissue can scarcely be made a satisfactory basis for ascribing acceleration.

[36] Biochem. Bull., 1913.

[37] Mellanby: Journ. Physiol., 36, p. 447.

Miescher[38] and Noël Paton[39] found for the fishes developing gonads at the expense of other tissues.

Anglas,[40] a student of insect metamorphosis, has suggested that phagocytes may act "lyocytically," that is, excrete an enzyme which may attack the tissues, causing atrophy. This suggestion is similar to the process of heterolysis of Jacoby; but in our present case, inasmuch as heterolysis and autolysis would give similar results, we find no evidence for this view.[41]

Summarizing, we may say that no evidence is deducible from the foregoing experiments tending to show that autolysis is accelerated in the atrophying tissues of the larval frog.[42]

[38] (L.c.)

[39] Paton, Noël: Fisheries Board of Scotland, 1898, p. 175.

[40] Comptes rendus, Paris (11), 51, p. 947; 52, p. 219; Bull. Sci. Fr. et Belg., 34, p. 363.

[41] In one regard, the process of lyositosis is different from heterolysis, for the former method implies that the leucocytes are alive, while heterolysis may occur *in vitro*, after the tissues are dead. Note the relation to excretion of leucoprotease.

[42] That the absorption of tissues which have suffered lesions does not involve acceleration of tissue enzyme action is substantiated by the graph shown above, where the rates of autolysis in muscles (1) normal and (2) with nerve cut for one week, are compared. It will be noted that the control and experiment proceed in a similar manner. The method used was as follows: Rabbit, etherized, sciatic cut in one leg; asepsis throughout; wound sewn up, iodine, bandage; after one week, muscles concerned were excised, weighed wet, ground in sand, made to given amount of distilled water and allowed to remain in thermostat at 40° C. for twenty days, tannic non-precipitable nitrogen being determined by Kjeldahl method at two day intervals. Results in cc. $\frac{N}{5}$ NH_3.

CONTRIBUTIONS TO THE PHYSIOLOGY OF THE STOMACH

XVIII. On the Sensibility of the Gastric Mucosa

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I. THE ABSENCE OF PAIN AND TACTILE SENSIBILITY IN THE NORMAL GASTRIC MUCOSA

Everyday experience tells us that the stomach mucosa is not sensitive to touch. Pawlow (15) states: "It can hardly be doubted that under normal conditions the surface of the stomach has a certain degree of tactile sensibility" (p. 90). The term tactile sensibility is evidently used here in the sense of a general response to mechanical stimulation rather than as implying a true tactile sensibility. When solid food is swallowed, no tactile sensation is felt after the food has passed the pharynx, unless the mass is so large that it causes unusual distension of the esophagus. In that case it may be felt all the way down the esophagus, but this is due to the distension of the wall of the esophagus and not a tactile sensation from contact with the mucosa.

Hertz (1) tested on himself the tactile sensibility of the mucosa of the esophagus by means of an esophagoscope with a slit down the side so that a metal bulb, in a long holder, could be moved along the mucous membrane of the esophagus and the pharynx. The pharyngeal mucosa was found to be sensitive to touch, the esophageal mucosa was insensitive

One of us (A. J. C.) tested the tactile sensibility of his own gastric mucosa, swallowing a good sized rubber rube, through which was passed a test tube brush attached to a strong piano

wire. Pulling the test tube brush about in the cavity of the stomach produced no sensation.

Numerous tests were made on the gastric mucosa of Mr. V., our gastric fistula case. Gently touching or striking the mucosa with blunt objects produces no sensation. If the mucosa is rubbed or pressed very vigorously with a blunt object Mr. V. says he "can feel it," he cannot describe the sensation thus produced except in a negative way. It is not like touch, nor is it pain or hunger (2). Whatever the character of the sensation may be we are satisfied that it is a real change in the stream of consciousness, for he recognizes these stimuli when he has no other means of knowing that the gastric mucosa is being handled. The sensation may not originate in the mucosa, but in the muscularis (tonus relaxation through reflex inhibition) or possibly in the visceral peritoneum, as the pressure must be considerable to produce it.

We are satisfied that the stimulation of the normal gastric mucosa of Mr. V. does not produce tactile sensation. The same conclusion has been reached by previous workers using human gastric fistula cases.

Gastric pain is a familiar clinical symptom, the hunger pangs, which are of gastric origin, are in reality hunger pains (2), and most of our readers have probably experienced the gastric pain produced by swallowing excessively hot food. Do any of these pains originate from stimulation of nerve endings in the gastric mucosa? We are satisfied from numerous tests on Mr. V. that *the sensation of pain cannot be produced from the normal gastric mucosa by any stimulation confined to the mucosa itself.* Pinpricks or incisions of the normal mucosa do not seem to affect consciousness in any way. It does not follow that pain may not be caused by the stimulation of the hypersensitive mucosa. We have no personal observations touching this point, but the literature seems to show that the gastric pain accompanying excessive inflation, gastric ulcers, and chronic obstruction is due to the mechanical stimulation of hypersensitive nerves or nerve endings in the muscularis or sub-mucosa by excessive distension or contraction.

II. THE SENSATION OF TEMPERATURE

In 1846 Weber suggested that the sensation of cold or warmth in the epigastrium, after drinking ice cold or very warm water, originates in the skin of the abdomen over the stomach and not in the stomach mucosa (3). According to Weber sufficient conduction takes place through the walls of the stomach and abdomen to stimulate the temperature nerves of the skin. Becher (4) swallowed a single rubber tube and through it injected water of different temperatures into the stomach. He did not experience any heat or cold sensation before the heat or cold has passed through the walls of the tube and stimulated the mucosa of the esophagus. Mueller concluded that ice water produced no sensation in the stomach. Zimmerman (5) irrigated his stomach with hot or cold water through a thick rubber tube. He claims that hot or cold sensations were felt only when the lower end of the tube was 30 to 35 cm. distant—hence not far from the lower end of the esophagus. He therefore concludes that the sensation is projected from the esophageal mucosa. Mackenzie (6) believes that the temperature sensations induced by hot and cold water into the stomach is due to reflex vaso-motor changes in the skin of the abdomen. Nystrom (7) reports that touching the gastric mucosa of a man with gastric fistula, with a hot piece of metal or piece of ice, does not call forth any definite temperature sensations. According to Hertz (1) the heat or cold sensations felt in the epigastrium, upon swallowing hot or cold water, comes from the lower end of the esophagus. The water accumulates here before the cardiac orifice opens and thus that part of the esophagus is stimulated more than the parts above. He warrants his conclusion by this observation: by auscultation over the epigastrium after swallowing a mouthful of very hot or cold water, one will find that immediately after the second deglutition sound, which occurs after the last trace of food has entered the stomach, the hot or cold sensation disappears. He also states that upon injecting hot or cold water through a double India rubber tube, no temperature sensation was noticed before three or four ounces had passed into the stomach. An ill-defined

temperature sensation was then experienced, but this was due to the conduction of heat or cold to the esophageal wall. He therefore concludes that the stomach mucosa is not endowed with heat or cold nerve endings. Quincke (8) introduced hot and cold water into the stomach of a boy with a gastric fistula and reports that the patient experienced vague heat or cold sensations. Neumann (9) and Roux (10) injecting hot and cold water into the stomach through a double rubber tube, experienced heat and cold sensations in the stomach, the cold sensation being the more pronounced. Head, Rivers and Sherren (11) injected water at different temperatures into the colon of a patient, upon whom a colostomy had been performed, and found that water at 20 to 40°C. produced no sensation at all but water at 50°C. and very cold water did give rise to temperature sensations, but that these sensations were by no means as pronounced and as easily localized, as those produced by applying water at the same temperatures to the skin. Head concludes that the viscera is endowed with protopathic temperature sensibility only.

It is evident from the above that by far the majority of those who have investigated this problem, have experienced a vague heat or cold sensation, in the region of the epigastrium, upon stimulating the stomach mucosa with very hot or very cold water. Those who do not believe that the sensation arises in the stomach mucosa explains its origin in one of the following ways:

(*a*) It is due to conduction to the skin of the abdomen.

(*b*) It is due to reflex vascular changes in the skin of the abdomen.

(*c*) It is projected from the esophagus.

(*d*) It arises in the lower part of the esophagus.

Our own experiments on Mr. V. and on ourselves go to show that the above mentioned theories are not tenable. That is to say, that the gastric mucosa is endowed with protopathic temperature sensibility.

The first series of experiments was on Mr. V. He was blindfolded and was not told the nature of the experiments. The water 40–60 cc. was injected through a small rubber tube which was passed through the large permanent tube of the fistula.

With water at 50°C., he said that he felt a heat sensation in the stomach after a latent period of about five seconds. When the same amount of water at 10°C. was injected a cold sensation was felt after a slightly shorter latent period. A metal rod 5 mm. in diameter heated to about 50°C. was passed down through the fistula catheter until the end touched the mucosa of the dorsal wall of the stomach. A longer latent period elapsed here before any heat sensation was noticed. When, however, a small piece of ice, held by a pair of forceps, was brought into contact with the stomach mucosa, the latent period was not much longer than when the cold water had been injected. A cold sensation was distinctly felt. These experiments were repeated over and over again and Mr. V. was invariably able to recognize whether the mucosa was being stimulated with hot or cold media, provided the hot media was 45 to 55°C. and the cold at least 13°C.

The temperature sensations initiated by touching the gastric mucosa directly with hot or cold solids cannot come from the stimulation of the esophageal mucosa. The latent period is too short to allow temperature conduction to the skin of the abdomen. But since it is possible that the cardia and the lower end of the esophagus of Mr. V. are patent, water injected through the fistula may reach the lower end of the esophagus. This possibility was guarded against in a second series of experiments, where we introduced the hot and cold water into a very delicate rubber balloon (condom) previously introduced into the stomach. The hot and cold water was correctly recognized as hot or cold, just as in the test with the water touching the mucosa directly, the only difference being a somewhat longer latent period. It is clear from the above that the gastric mucosa of Mr. V. is endowed with protopathic temperature sense.

A third series of experiments were carried out on ourselves. Three rubber tubes of suitable size were placed one inside the other so as to have three walls of rubber and two air spaces between the liquid in the inside tube and the esophageal mucosa, for the purpose of retarding heat conduction. The inside tube had a diameter of 3 mm. The subject was blindfolded and

50 cc. of water at 50°C. was injected into the stomach. A heat sensation was noted about ten to fifteen seconds after the injection, and about 60 seconds before enough conduction had taken place to cause the tube to feel warm in the mouth and throat. When the same amount of water at 10°C. was injected, a quite definite cold sensation was felt from five to ten seconds after the injection had commenced and about 60 seconds before the tube began to feel cold to the fingers or to the throat.

Both of us experienced heat and cold sensations and never made the mistake of confusing the heat sensation with the cold sensation. Confirming Neumann and others, we found the cold sensation to be more distinct and better localized than the heat sensation.

This certainly does not seem to indicate that the stomach mucosa is devoid of temperature sensations, nor that this sensation is projected from the esophagus, as has been suggested by a number of investigators. However, there is a possibility that some of the water might reach the wall of the lower end of the esophagus by being forced up along the tube. In view of the small amount of water necessary to evoke the heat and cold sensation and in view of the short latent period mentioned above, this seems very unlikely.

To make sure that the sensation did not arise as a result of stimulation of the esophagus, as well as to prove that the sensation is not due to conduction through to the skin of the abdomen, or to reflex vascular changes in the skin of the abdomen, a fourth series of experiments was performed. For these experiments an India rubber tube 1 cm. in diameter was used. Inside this large tube we placed two smaller tubes side by side; one of these was 5 mm. in diameter and the other was 3 mm. The smaller of these two tubes was not pulled clear through the outside tube, but only so far that its lower end was about an inch above the lower end of the outside tube. The 5 mm. tube was pulled through the outside tube so that it extended about 10 cm. below the lower end of it. A small glass tube 5 mm. long was now forced up into this longer tube as far as to the lower

end of the outside tube. The lower end of the outside tube was then tied securely about the longest tube at this point. This apparatus was now swallowed far enough down, so that the lower end of the outside tube reached just through the cardiac orifice. The longest tube, the end of which of course was open, therefore extended far down into the stomach. The smallest tube, whose lower end was within an inch of where the outside tube was tied, that is, within 2–3 cm. of the cardiac, was now connected with a pressure bottle. It had previously been found that hot water in passing through a glass tubing and connections of the pressure bottle lost approximately 8°C., and that water placed in the bottle at 10° was raised to 12°C. in passing through. Water at 58°C. (1) was now placed in the pressure bottle and permitted to flow down through the smallest inside tube. It, naturally, was forced up again between and around the two inside tubes. By so doing the heat was conducted through the wall of the outside tube and stimulated the heat nerve endings in the esophagus, so that a definite heat sensation was felt along its whole course, as well as in the mouth and pharynx. The striking feature about this, however, was that, although the walls of the lower end of the outside tube naturally became hot before those of the upper, the heat sensation was first felt in the mouth and throat and then gradually travelled down the whole length of the esophagus. At all times, however, the heat sensation was more intense in the throat and the upper part of the esophagus than in its lower end. While the walls of the esophagus were thus being stimulated, 50 cc. of water at 50°–55°C. was injected into the stomach through the longest inside tube which opened into the stomach and, after a latent period of about ten seconds, a spreading heat sensation was felt lower down than that resulting from the stimulation of the esophagus. The sensation also seemed better localized than that from the esophagus so that it was not very difficult to keep the two sensations apart in consciousness. The reason for the better localization might, of course, be due to the fact that more nerve endings were stimulated in the stomach mucosa, as the water came into direct con-

tact with it than was the case in the esophagus, where probably the rubber tube did not come into contact with the mucosa at all points.

When water at 10° was placed in the pressure bottle—the outside tube stimulating the esophagus thus being irrigated by water at 12°—and 50 cc. of water at 12° was injected into the stomach, a quite distinct, spreading, cold sensation was felt lower than that resulting from the stimulation of the esophagus. There was no difficulty in recognizing two separate and distinct cold sensations, one coming from the esophagus and one from the stomach.

While the esophagus was being stimulated by water at 12°C. in this tube system, a water bottle which was filled with water at 12°C. was placed on the skin of the abdomen. The two cold sensations resulting could easily be kept apart in consciousness. 50 cc. of water at the same temperature was then injected into the stomach and a third distinct, spreading, cold sensation was felt in the stomach. This experiment was repeated several times with hot and cold water as indicated above and with the same results.

If the temperature sensation felt, upon stimulating the stomach mucosa, is due to conduction through to the skin of the abdomen, or to reflex vascular changes in the skin of the abdomen, or is projected from the esophagus, how is it possible that one can distinguish in consciousness the sensations coming from the esophagus, the stomach, and the skin, at the same time, when each is being stimulated by water at the same temperature, as shown above?

To make sure that the sensation was not due to conduction through to the skin of the abdomen, the bulb of a thermometer was pressed close to the skin over the stomach and covered with absorbent cotton. After it had become stable, 200 cc. of water at 10° was injected into the stomach and the mercury in the thermometer watched for a period of five minutes. This was repeated several times. In each case, after a latent period of five minutes, the mercury had fallen only a small fraction of a degree. It is therefore evident that the cold sensation which one feels

after a latent period of from five to ten seconds, after the injection of 20 cc. of cold water into the stomach, is not due to conduction through the abdominal wall and stimulation of the nerve endings in the skin.

We agree with Hertz that when one swallows a mouthful of ice water, the most intense cold sensation seems to come from the lower end of the esophagus, and that this is undoubtedly due to the fact that the water accumulates there and is detained for a shorter or longer period before the cardiac orifice opens so that it can flow into the stomach. He further states that the cold sensation disappears after the water has entered the stomach. This we cannot corroborate. We invariably feel a vague spreading cold sensation after the water has entered the stomach, but which disappears after a few seconds. Even if it were the case that no cold sensation could be felt after the water had passed into the stomach, this could not prove that the stomach mucosa is devoid of heat and cold nerve endings. In the first place, a mouthful of cold water in passing down into the stomach, is warmed sufficiently to raise its temperature several degrees. On this account the nerve endings in the mucosa of the stomach are not stimulated as intensely as are those in the esophagus. The empty stomach contains 10–50 cc. of fluid at 38°C. which rapidly mixes with and thus raises the temperature of the swallowed water. It is also well known that stronger impulses, reaching the central nervous system from one part, tend to suppress in consciousness weaker impulses of the same nature, reaching it from some other part, so that it is difficult to separate the two sensations in consciousness.

From our experiments we conclude:

(*a*) The stomach mucosa is endowed with heat and cold nerve endings.

(*b*) These fibers are, as Head suggests, of the protopathic type.

(*c*) They are more abundant, or more readily stimulated in the throat and the esophagus than in the stomach.

III. THE GASTRIC COMPONENT OF THE SENSATION OF APPETITE

New facts touching the relation of the sensations of hunger and appetite, and particularly the peripheral sensory apparatus for hunger, have been reported from this laboratory in a previous paper (2). It was shown that the hunger sensation cannot be produced by any kind of stimulation of the nerve endings in the gastric mucosa. In the normal empty stomach the hunger sensation is initiated by strong contractions of the stomach wall, and by these contractions only. It was shown that these contractions, no matter how they are initiated, give rise to the characteristic sensations of hunger pangs. It was therefore concluded that the gastric sensory apparatus for the hunger sense lies in the muscular coats or in the connective tissue, but not in the mucosa. The hunger sense is a complex of kinestetic sensation (tension) and pain. Continued investigations on Mr. V., on ourselves, and on a number of other persons here in our laboratory have served to confirm the above conclusion. When an individual can clearly recognize the pangs of hunger he also recognizes (in suitable experiments) that these sensations are not induced by stimulation of the gastric mucosa.

Pawlow (15) concludes that "the tactile sensation of the stomach at the moment of entry of food is capable of awakening or increasing appetite" (p. 91). In the experiments cited by Pawlow in support of this conclusion the stimulation of the nerve endings in the mouth and in the esophagus was not excluded. However, the theory that stimulation of the normal gastric mucosa contributes to the sensation of appetite is clearly established by the experiments reported below.

One of us (A. J. C.) working on himself soon recognized that stimulation of the nerve endings in the gastric mucosa modified the flow of consciousness, although this modification did not consist in the sensation of hunger. This can readily be experienced by anyone who is sufficiently interested to try, by introducing moderately cold water, beer, wine, weak acids (0.5 per cent) or weak alcohol through a tube into the stomach so as to avoid stimulation of nerve endings in the mouth and esophagus.

The sensation produced by these substances in the stomach is rather transitory, but may persist for several minutes. With the exception of cold water which is also felt as cold, these various substances give rise to a characteristic sensation which fuses with, or cannot be distinguished from appetite. It is like the sensation of increased appetite experienced by most people at the beginning of a meal after eating a few morsels of palatable food. The sensation is pleasant, and invariably turns the attention towards food and eating.

We are accustomed to think that the above substances affect consciousness solely through stimulation of nerve endings in the mouth. This view is no longer tenable. By introducing these substances through the stomach tube at the height of a gastric hunger contraction one actually experiences a *successive contrast of the sensations of hunger and appetite*, as these substances temporarily inhibit the hunger contractions in stimulating the gastric mucosa. This method was demonstrated before the Federation of American Biological Societies in December last year (12). The experiment has been repeated a number of times on the junior author. From the first it was clear that, when beer, cold or hot water, were introduced into the stomach during a vigorous hunger contraction, the sensation resulting was the exact opposite of that caused by the hunger contraction. In place of an unpleasant, tense sensation, associated with restlessness, the sensation caused by these different stimuli is one of relief. A pleasant tingling sensation is felt in the stomach. One feels perfectly at ease, but the thoughts tend to revert to the dinner table. At first he was not able to say just what this sensation was like, although it was a familiar one. After paying close attention to the sensation experienced at meals just after a few mouthfuls of good food or drink have been swallowed, he became convinced that the two sensations are very much alike if not identical.

How do we know that the sensation temporarily produced by the above substances in the stomach is directly due to stimulation of nerve endings in the gastric mucosa? It has already been shown that the introduction of these substances in the

stomach temporarily inhibit the gastric tonus and the gastric hunger contractions (13). May not the sensation be one of negative character, so to speak, that is, diminution or absence of hunger? We are in position to answer this question definitely in the negative. In the first place, the sudden and spontaneous relaxation of the stomach at the end of a period of gastric hunger contractions is accompanied by a characteristic sense of relief and disappearance of a certain tension or unpleasant mental stress, but this sensation complex has not the positive character that directs attention to food and eating. It is essentially relief from pain. Secondly, putting these substances directly into the stomach which is quiescent and very greatly relaxed, still inaugurates this temporary appetite or appetite-like sensation. Hence we conclude that it is directly induced by stimulation of certain nerve endings in the gastric mucosa itself. Of course, if the nerve endings in the gastric mucosa are thus stimulated at the time the muscularis is in strong tonus and hunger contractions, the appetite-like sensation is fused with that of relief from the pangs of hunger.

It is significant that normal human gastric juice having full acid strength (0.45–0.50 per cent free HCl) is capable of inducing this sensation from the stomach. This has been verified repeatedly on one of us (A. J. C.) by introducing 50 cc. of appetite gastric juice of Mr. V. through the stomach tube. Gastric juice of weaker acidity (0.20 per cent) does not have this effect. As this full strength gastric juice is rapidly secreted into the stomach at the beginning of eating, it is probably a factor in the augmentation of appetite by the very act of eating. It may be pointed out that the above facts permit us to see a suggestion of truth in Beaumont's theory that turgescence of the gastric glands is the cause of hunger. In the first place, Beaumont, in common with most physiologists, did not clearly distinguish between hunger and appetite but used the two terms interchangably. If we, then, substitute appetite for hunger, we see that with the stomach normal the appetite sensation may be actually initiated or augmented by gastric juice, not through me-

chanical pressure of the juice in the ducts, but by acid stimulation of nerve endings in the gastric mucosa.

It need scarcely be pointed out that when foods or liquids are taken into the mouth and swallowed in the normal way their main influence on appetite is via nerve endings in the mouth. In fact, the latter is so predominant that only by excluding it are we able to clearly distinguish the gastric factor. The memory factor in appetite is therefore preëminently gustatory and olfactory.

We have as yet no device for recording objectively the *quality* of a *sensation*. In the preceding pages we have detailed certain conditions under which certain sensations are produced. We have called these sensations hunger and appetite, respectively, in accordance with our best efforts of subjective analysis. The experimental conditions are so simple that any biologist with a little perseverance can duplicate them. We believe they will verify our conclusions that

1. Hunger and appetite are qualitatively different sensations.
2. The sensory apparatus for hunger is distributed in the stomach wall and is stimulated by a certain type of contractions of the empty stomach.
3. The gastric mucosa is endowed with a protopathic appetite sensibility.

IV. THE SENSATION OF FULNESS

All who have studied the origin of the sensation of fulness seem to agree that it does not originate in the mucosa of the stomach, and our own results agree with this view. The literature is extensively reviewed by Hertz (1).

Hertz and his co-workers inflated the stomach of two healthy men with air through a tube connected with a manometer. They found that a "sensation of fulness or tightness in the upper part of the abdomen associated with a desire to eructate, was felt as soon as the intragastric pressure reached respectively 12 and 14 mm. of mercury in the two cases. The pressure fell after 20 seconds by approximately 2 mm., owing apparently to relaxa-

tion of the tone of the stomach, and simultaneously the sensation of fulness disappeared. Now on slowly injecting more air, the pressure gradually arose to its original height and the sensation reappeared; it again disappeared after 20 seconds, the pressure simultaneously falling 2 mm., after which it remained constant. Exactly the same rise in pressure and the same sensation of fulness, followed by a fall in pressure and a disappearance of the sensation, were produced four times in succession by injecting air, none being allowed to escape in the interval. These observations proved that the tension exerted from within on the circular muscle fibers of the stomach is the cause of the sensation of fulness."

That this is the main source of its origin seems quite clear. There is a possibility that the stretching of the abdominal muscles as well as the pressure on other structures in the abdominal cavity contribute to the sensation of fulness. As a proof that this element has little if any significance in giving rise to the sensation, Hertz related experiments on persons with atonic stomachs. When six grams each of sodium bicarbonate and tartaric acid are swallowed separately 1700 cc. of carbon dioxide are given off under atmospheric pressure at body temperature. This invariably causes an unpleasant and sometimes painful sensation of fulness in normal individuals while in persons with atonic stomachs it does not cause any sensation of fulness. Hertz therefore concludes that "the sensation of fulness in the stomach is due to tension on its muscular coat, and depends very little and only in extreme cases on the stretching of the abdominal wall."

To these observations of Hertz we can add our negative findings in regard to the gastric mucosa as a contributing factor. Chemical or mechanical stimulation of the mucosa (whether the stomach is in strong or in feeble tonus) never produces a sensation similar to that of fulness. At the same time, it must be noted that mere tension on the muscular coats, that is, intragastric pressure, will not under all conditions give rise to the sensation. The degree of intragastric pressure required to cause a feeling of fulness according to Hertz, is frequently ex-

ceeded at the height of a period of hunger contractions of the empty stomach, when a distended balloon is in the stomach; yet, the sensation referred to the epigastrium in these conditions is that of emptiness, not fulness. It is therefore clear that a *certain amount of tonus relaxation of the stomach must be present before tension or pressure on the walls of the stomach produce the sensation of fulness.*

V. THE EFFECT OF STIMULATING THE GASTRIC MUCOSA IN MAN ON THE VASO-MOTOR CENTER AND THE REFLEX EXCITABILITY OF THE SPINAL CORD

Effect on the Vaso-motor Center

Two series of experiments were performed (on L. H. B.), the first series with the subject awake and the second during sleep. The same method of experimentation was followed as that used by one of us in the experimentation on Mr. V. to determine the effect of the hunger contractions on the activity of the vaso-motor center (13). The man experimented on was blindfolded and endeavored to relax as completely as possib e.

In the first series, when relaxation was most comp ete, the same rhythm of the vaso-motor tone was noted synchronously with the strong hunger contractions as were observed on Mr. V., namely, an increase in the size of the arm during the strong gastric contraction. It was rather difficult, however, to become so completely relaxed that this phenomena could be seen.

When cold or hot water was introduced into the stomach through the tubes in quantities of 50–100 cc. at a time there was nearly always a shrinking of the arm, that is, vaso-constriction (fig. 1). Water at body temperature did not seem to affect the tonus of the vaso-motor center. Water at 10°C. caused less vaso-constriction than water at 55°C.

In order to ascertain whether this reflex was due to the influence on the vaso-motor center by conscious processes, similar experiments were performed while the subject was asleep. It is not an easy matter to go to sleep with a plethysmograph on one arm and two rubber tubes in one's throat and esophagus. But,

after a walk of about twenty miles followed by a long swim, it was possible. The same rhythmic variation in the vaso-motor tone was now observed as was seen while awake but perfectly relaxed. There was a gradual increase in the size of the arm as the tonus of the stomach increased. This increase or vaso-dilation was greatest when the stomach contraction was at its highest and the size of the arm decreased as the stomach relaxed. From this it seems evident that this activity of the vaso-motor center is not due to conscious processes.

Moderately hot or moderately cold water in the stomach did not seem to affect the activity of the vaso-motor center during

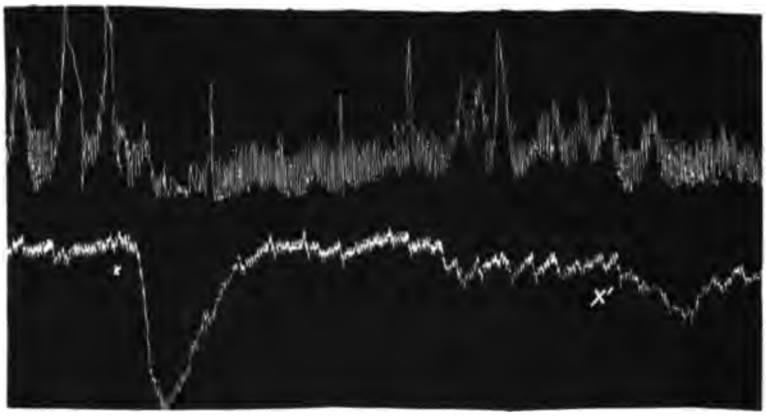

Fig. 1. Upper tracing from the empty stomach of L. H. B.; lower tracing from arm plethysmograph. X, introduction of 100 cc. water at 50°C. through double stomach tube into stomach; x', introduction of 100 cc. water at 5°C. in the same way into the stomach. Showing reflex vaso-constriction from temperature stimulation of the gastric mucosa.

sleep. Very cold water either did not have any marked effect, or else the stimulation was sufficient to awake the subject. Warm water did not cause a marked vaso-constriction unless the subject woke up as a result of the injection. The marked vaso-constriction observed when the stomach mucosa is stimulated with very cold or very warm water therefore seems to be due mainly to the effect of conscious processes on the vaso-motor center.

Effect on the Reflex Excitability of the Spinal Cord

The hunger contractions of the empty stomach cause an increase reflex excitability of the cord (13). Similar effects are obtained on stimulation of the nerve endings in the gastric mucosa. Hot or cold water introduced directly into the stomach by the tube so as not to stimulate the mouth and esophagus cause a temporary augmentation of the knee jerk (fig. 2). The increase caused by the hot water (50°C.) is a trifle greater than that caused by the cold water. Practically the same results were obtained on both of us. No attempt was made to study the influence on the knee jerk of stimulation of the gastric mucosa during sleep.

a *b*

Fig. 2. Record of knee jerk of A. J. C. *a*, 50 cc. water at 50°C. introduced into stomach through double tube; *b*, 50 cc. water at 5°C. into stomach. Showing augmentation of reflex excitability of spinal cord from temperature stimulation of the gastric mucosa.

SUMMARY

1. The normal gastric mucosa is devoid of pain and tactile sensibility, but protopathic temperature sensibility is present.
2. Chemical (and possibly mechanical) stimulation of nerve endings in the normal gastric mucosa gives rise to a sensation identical with appetite. The stomach mucosa may be said to be provided with a protopathic appetite sense.
3. The stimulation of the gastric mucosa does not contribute to the sensations of hunger and of fulness.
4. Stimulation of the gastric mucosa increases the reflex excitability of the spinal cord, and induces changes in vaso-motor tonus. The latter appears to be dependent on conscious cerebral processes induced by the gastric stimulation.

(1) Hertz: The Sensibility of the Alimentary Canal, London, 1911.
(2) Carlson: This journal, 1913, xxxi, p. 175.
(3) Weber: Wagner's Handworterte d. physiol., viii, p. 497.
(4) Becher: Zeitschr. f. Psychol., 1908, xlxi, p. 341.
(5) Zimmerman: Mitteil a. d. Gernzg. Med. u. Chir., 1909, xx, p. 445.
(6) Mackenzie: Symptoms and their Interpretations, 1909, p. 125.
(7) Nystrom: Mitteil. a. d. Grenzg., Med. u. Chir., 1909, xxi, p. 125.
(8) Zuincke: Arch. f. exper. Pathol. and Pharm., 1889, xxv, p. 375.
(9) Neumann: Wien kl. Woch, 1905, xix, p. 923.
(10) Roux: Maladies du Tube digestive, Paris, 1907, p. 375.
(11) Head, Rivers and Sherren: Brain, 1905, xxviii, p. 99.
(12) Carlson: This journal, 1914, xxxiii, p. xv.
(13) Carlson: This journal, 1913, xxxii, p. 245.
(14) Carlson: This journal, 1913, xxxi, p. 318.
(15) Pawlow: The work of the Digestive Gland, London, 1902.

VARIATIONS IN IRRITABILITY OF THE REFLEX ARC

II. Variations Under Strychnine

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In previous papers[1] I have described the procedure by which the Martin method of measuring Faradic stimuli has been applied to a study of the variations in reflex irritability in the spinal cat. The fluctuations in threshold reported as occurring in asphyxia were large, the rise in threshold on withdrawing air amounting usually to more than 600 units from an initial threshold of about 5 units. The method permits equally well and with great accuracy the detection of changes of much less magnitude, down to a few hundredths of a unit in a good spinal preparation. On this account it was thought feasible to attempt the study of influences which would be expected to lower the threshold instead of raising it, although the changes on a reflex of low threshold would necessarily be small. The flexion reflex was used as in previous studies and was elicited by stimulation of the central end of the cut tibial nerve. In most experiments I have also followed the alterations in threshold of the crossed extension reflex and of reflex extension of the fore-limb. These two reflexes were elicited by single break shocks to the same nerve which gave flexion, namely, the tibial. As a control the irritability of a nerve muscle preparation on the fore-limb was determined at frequent intervals during some of the earlier experiments. The movement here was extension at the wrist, caused by stimulation of the peripheral end of a branch of the radial nerve. The experimental procedures adopted were never found to affect the threshold of this nerve-muscle preparation and in the later experiments

[1] E. L. Porter: This Journal, 1912, xxxi, p. 141, and 1913, xxxi, p. 223.

of the series its threshold was no longer followed. In all measurements on the flexion reflex the smallest detectable record of movement on the drum was taken to indicate that the threshold had been reached. When crossed extension and extension of the fore-limb appeared the movement, if any, was always comparatively large, hence to determine when the threshold had been reached they were observed directly without record on the drum. A 0.1 per cent solution of strychnine sulphate was the "excitant" used and the injections (generally 0.1 or 0.2 mgm. strychnine) were made into the jugular vein at intervals of from 2 to 10 minutes until the animal exhibited violent convulsions. It was never found possible to obtain reflex extension of the fore-limb before strychnine was given, although stimuli as high as 780 Z units have been applied in some cases. Crossed-extension was readily elicitable but its threshold in the undrugged animal, although fairly constant in any given case, varied widely in different preparations, running from a threshold approximately twice that for flexion to one a hundred times as high.[2]

The literature describes the action of strychnine as that of an excitant to the central nervous system. "The alkaloids of the strychnine group have a powerful stimulant action on the central nervous system, especially on the spinal cord."[3] "Die typische Wirkung aller dieser Alkalöide (strychnine group) besteht in der hochgradigen Steigerung der Reflexerregbarkeit . . . . und findet ihren Ausdruch in dem Tetanus." "Die Krampfanfälle werden . . . . durch die kleinsten, oft gar nicht mehr nachweisbaren Reize hervorgerufen, so dass sie scheinbar ohne alle Veranlassung eintreten."[4] "Die characteristische Wirkung des Strychnins sind die Steigerung der Reflexerregbarkeit und die Krampfanfälle, welche schliesslich zum Tode führen."[5] Sollmann[6] speaks of "increased irritability" and "increased excitability" in describing the action of the drug.

[2] Cf. Exp. of Nov. 15, Table I, p. 174 and Exp. of Dec. 6, Table II, p. 177.

[3] Cushny: Pharmacology and therapeutics, Philadelphia, 1901, p. 190.

[4] Schmiedeberg: Grundriss der Pharmakologie, Leipzig, 1906, pp. 108, 109.

[5] Magnus: Article "Strychnin" in the Real-Encyclopädie der gesamten Heilkunde, 4th edition, Berlin, 1907 and following.

[6] Sollmann: A Text-book of pharmacology, Philadelphia, 1908, pp. 143 and 876.

Storm van Leeuwen[7] using the flexion reflex in the spinal cat gave repeated stimuli of constant strength and noted the increased height of contraction following strychnine. He referred to this as "Steigerung der Reflexerregbarkeit." Just what is meant by heightened reflex irritability is not stated so definitely by other authors. They obviously intend to include the widespread and convulsive responses following strychnine and in addition they give the impression that impulses which in the unpoisoned animal are denied entrance to the cord are enabled after exhibition of strychnine to penetrate and produce reflex effects.

In 9 out of 15 experiments of my series in which the results were clear-cut the expected lowering in reflex threshold did occur. Following is a protocol of such an experiment.

Experiment of November 15, 1913

ELAPSED TIME	PROCEDURE AND REMARKS	THRESHOLDS IN Z UNITS		
		Flexion	Crossed extension	Extension of fore-limb
0.35		2.4		Inelicitable
0.45			5.2	Inelicitable
1.08		2.5	5.8	Inelicitable
1.22	Liquid removed from electrodes			
1.23		1.8	5.8	Inelicitable
1.37	0.1 mg. strychnine			
1.40		1.3	3.4	Inelicitable
1.44		1.2	2.1	Inelicitable
1.47	0.1 mg. strychnine			
1.49	Violent convulsions			
1.51		1.6	2.3	2.6
2.03		1.6	2.5	
2.05	0.1 mg. strychnine			
2.10		1.5	3.4	
2.12			1.6	1.6
2.14		1.4	1.8	
2.16				2.2

[7] W. Storm van Leeuwen: Archiv für die gesammte Physiologie, 1913, cliv, p. 307.

After one injection of strychnine a minimum of 1.2 Z units was reached from an initial threshold of 2.4 Z units, a drop of 50 per cent. The crossed extension reflex, starting with a threshold of 5.2 Z units reach 1.6 Z units, nearly the same minimum as for flexion. Reflex movement of the fore-limb also reached 1.6 Z units. The entire series of 9 experiments in which a drop in threshold occurred is summarized below.

TABLE I

Summary of experiments in which a drop in threshold of the flexion reflex occurred following intravenous injections of strychnine. Thresholds are in Z units

DATE	REFLEX	THRESHOLD AT START	LOWEST THRESHOLD REACHED AFTER STRYCHNINE	THRESHOLD AT END OF EXPERIMENT	PERCENTAGE LOWERING OF THRESHOLD
March 17, 1913	Flexion	3.2	2.3	2.9	28
March 18, 1913	Flexion	5.3	4.4	6.1	17
June 6, 1913	Flexion	5.1	4.1	6.6	20
June 28, 1913	Flexion	3.3	2.3	2.4	30
July 8, 1913	Flexion	3.6	1.6	2.2	56
	Crossed-extension	28.8	1.8	2.7	94
October 25, 1913	Flexion	3.5	1.9	3.7	46
November 15, 1913	Flexion	2.4	1.2	1.4	50
	Crossed-extension	5.2	1.6	1.8	69
	Fore-limb extension		1.6	2.2	
November 28, 1913	Flexion	2.5	2.3	4.0	8
	Crossed-extension	4.5	3.3	8.4	26
	Fore-limb extension		5.4	7.6	
January 10, 1914	Flexion	4.1	2.8	3.4	31
	Crossed-extension	196.0	3.0	3.9	98
	Fore-limb extension		3.0	6.2	

But the lowering of the flexion reflex threshold after strychnine quite distinctly failed to occur in the remaining 6 of the 15 experiments. In the following protocol (abridged from the original) is an example of this result.

Following the injections of strychnine the threshold never went below 2.3 Z units, 0.7 of a unit above the initial value, despite the continuance of injections until convulsions occurred.

But although the flexion threshold was not lowered the drug was strongly operative on the thresholds of other reflexes. Crossed-extension was lowered from an initial threshold of 36

Experiment of November 30, 1913

ELAPSED TIME	PROCEDURE AND REMARKS	THRESHOLDS IN Z UNITS		
		Flexion	Crossed extension	Fore-limb extension
0.35		1.6	36.0	Inelicitable
1.04		3.2		Inelicitable
	Electrodes dried			
1.14		1.7	5.8	Inelicitable
1.20		2.1	7.2	Inelicitable
1.25	0.1 mg. strychnine			
1.30		2.3	5.7	Inelicitable
1.34		2.6		Inelicitable
1.35	0.1 mg. strychnine			
1.36	Vigorous twitchings			
1.38		2.6	4.0	Inelicitable
1.45		3.3		Inelicitable
	Electrodes dried			
1.47		2.1	5.0	Inelicitable
1.49	0.1 mg. strychnine			
1.50 to 1.53	Violent convulsions at intervals			
1.55		3.0	8.4	Inelicitable
1.59	Convulsions			18.5
2.01	Electrodes dried			
2.03		2.3	7.3	9.8
2.21	0.1 mg. strychnine			
2.23		2.2	2.8	4.3
2.26	0.2 mg. strychnine			
2.26	Violent convulsions			
2.27		2.2	3.0	
2.32	0.2 mg. strychnine			
2.33		2.2	2.3	2.6
2.37	0.2 mg. strychnine			
2.40		2.3	2.4	2.6
2.45		2.2	2.5	2.4

Z units to a minimum of 2.3 Z units. Extension of fore-limb, inelicitable at the beginning of the experiment, was finally lowered to a threshold of 2.4 Z units, after repeated injections of

strychnine and after the animal had been exhibiting convulsions for nearly an hour. The thresholds of the three reflexes were practically identical at the close of the experiment, flexion 2.2, crossed-extension 2.5 and extension of fore-limb 2.4. Here obviously the "heightened irritability" was not due to the greater ease with which stimuli entered the cord but to the greater ease with which such impulses elicited reflexes not normally thrown into activity by the particular group of sensory neurons stimulated. In some cases the thresholds of the three reflexes became actually identical for several readings, as the following protocol shows. Note the close correspondence of readings taken at 1.10, 1.17 and 1.20.

Experiment of January 17, 1914

ELAPSED TIME	PROCEDURE AND REMARKS	THRESHOLDS IN Z UNITS		
		Flexion	Crossed extension	Fore-limb extension
0.45		1.8	5.9	Inelicitable
0.47		1.9		Inelicitable
0.48	0.4 mg. strychnine			
0.48	Violent convulsions			
0.52		1.9	2.6	Inelicitable
0.56				3.0
0.59		2.3	2.6	2.6
1.02		2.2		
1.04			2.5	2.5
1.05	0.4 mg. strychnine			
1.10		2.4	2.4	2.4
1.17		2.6	2.6	2.6
1.20		6.5	6.5	6.5

The results seem to prove that synaptic resistances throughout the cord have been equalized. That this proof is not rigid will be indicated later (p. 182).

A summary of the 6 experiments in which no drop in reflex threshold was found follows (Table II). The lowest flexion threshold reached after strychnine is seen to vary from 0.1 to 2 Z units above the threshold in the undrugged animal.

A difficulty frequently encountered in determining thresholds was the short circuiting of a part of the current by blood or

lymph collecting between the electrodes. This necessitated a stronger current than was the case after the liquid had been removed. In the experiment of November 15 (p. 173) this was the case. At 1.22 liquid was removed from the electrodes and the threshold dropped from 2.5 Z units to 1.8 Z units. Similarly in the protocol of November 30 (p. 175), the threshold was lowered at 1.14, 1.47, and 2.03. Conceivably, then, a lowered threshold

TABLE II

Summary of experiments in which no drop in threshold of the flexion reflex occurred following intravenous injections of strychnine.
Thresholds are measured in Z units

DATE	REFLEX	THRESHOLD AT START OF EXPERIMENT	LOWEST THRESHOLD REACHED AFTER STRYCHNINE	THRESHOLD AT END OF EXPERIMENT	PERCENTAGE RISE IN THRESHOLD
June 7, 1913	Flexion	3.6	5.6	7.2	52
June 30, 1913	Flexion	1.4	1.5	1.5	7
November 30, 1913	Flexion	1.6	2.1	2.2	31
	Crossed extension	36.0	2.3	2.5	
	Fore-limb extension		2.4	2.4	
December 6, 1913	Flexion	2.8	3.2	3.7	14
	Crossed-extension	300.0	5.2	94.8	
	Fore-limb extension		5.2	94.8	
January 7, 1914	Flexion	2.9	3.0	5.3	3
	Crossed-extension	5.6	3.0	5.9	
	Fore-limb extension		4.5	7.0	
January 17, 1914	Flexion	1.8	1.9	6.5	6
	Crossed-extension	5.9	2.4	6.5	
	Fore-limb extension		2.4	6.5	

following strychnine might commonly be masked by the presence of liquid between the electrodes. This has been shown to be improbable in an experiment in which liquid was prevented from accumulating on the nerve by a small pledget of cotton between the electrodes and in contact with the nerve. Immediate injections of strychnine were followed promptly by convulsions, but the threshold after strychnine stood 1.0 Z unit above its initial value. It was conceivable that the cotton had dried the

nerve and rendered it less irritable. The cotton was therefore wet with saline and left for five minutes. It was then removed, the surface of the nerve dried, and the threshold again determined. It was still 0.2 Z unit higher than before strychnine. The experiment was performed rapidly, only 22 minutes elapsing between the first and the final determinations. The conclusion seems justified that thresholds of the flexion reflex in the spinal cat frequently occur which cannot be lowered by strychnine.

That this conclusion holds also for the spinal frog is indicated by results obtained by Elrington.[8] He secured reflex contractions of the gastrocnemius muscle by stimulation of the ninth dorsal nerve root and measured the threshold before and after strychnine. His measurements are given in the units of the Kronecker scale and the strength of the primary current is not stated so that his results and my own are not comparable directly; percentage changes, however, can be compared. In eight out of twenty cases he found either no change in threshold after strychnine or a rise varying from 55 to 900 per cent of the initial value. I found in the cat in six out of fifteen cases a rise of from 3 to 52 per cent (Table II). In twelve cases he found a drop of from 17 to 95 per cent. I found similarly in nine cases a drop of from 8 to 56 per cent. Percentage alterations were thus much greater with his animals than with mine, but the proportion of cases in which strychnine failed to cause lowering was the same as I found, namely one out of three. Provided he used a primary current of the same strength in all cases his animals also show wider variations in normal threshold, that is, before strychnine. His values for initial thresholds vary from 60 to 2000 units on the Kronecker scale; mine from 1.4 to 5.3 Z units, Martin calibration (Tables I and II). Such wide variations are like those I found for the crossed-extension reflex, namely, 4.5 to 300 Z units (Tables I and II). This suggests that in using the reflex contractions of the gastrocnemius, Elrington may have been dealing with a reflex less simple than flexion or else the simplest reflexes in the frog have less uniform thresholds than corresponding ones in

[8] Elrington: Zeitschrift für allgemeine physiologie, 1914, xvi, p. 115.

the cat. It would appear also from Elrington's results that reflex contractions of the gastrocnemius vary in threshold from moment to moment for he frequently gives the value as "0–30," "100–200," and so forth. I have always found the threshold for flexion in the spinal cat very sharp and easy of determination, with an error of perhaps 1 or 2 per cent.

If the flexion reflex threshold be raised by ether, strychnine promptly lowers it. Figure 1 is a graphic record of such an experiment. Ether was administered by shunting a part of the air used for artificial respiration through an ether bottle. The

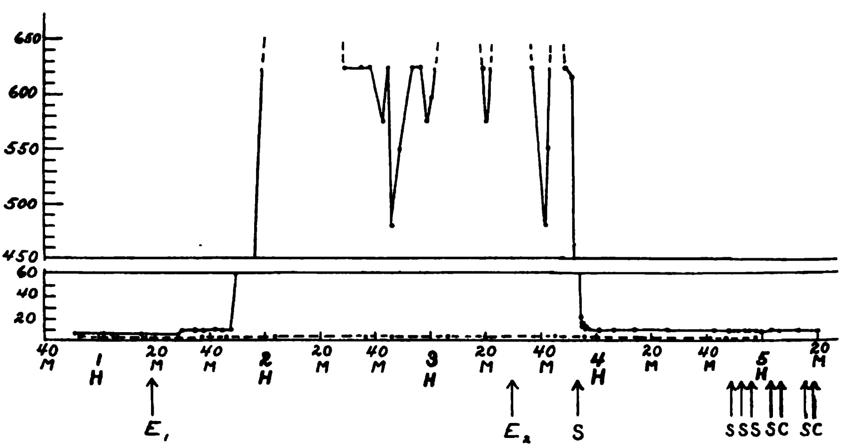

Fig. 1. Effect of injections of strychnine on a reflex threshold which had been raised by ether. E_1, administration of ether in varying amounts begun; E_2, amount of ether administered was left unchanged from this point to the end of the experiment; *S*, injections of strychnine of 0.2 mgm. or 0.3 mgm.; *C*, convulsions. Ordinates, values of *Z*; abscissae, elapsed time in hours and minutes. Continuous line, reflex; broken line, nerve-muscle preparation. A portion of the vertical part of the record from ordinate 60 to ordinate 450 has been omitted (Exp. of July 22, 1910).

proportion of ether to air could be varied at will. The original purpose was to raise the threshold only moderately, to an "ether plateau" so to speak, and hold it at that point for a considerable interval during which the strychnine could be injected. Regulating the administration of the ether so as to being this about was found impossible; the threshold either remained low or rose

to a great height and even suddenly and completely disappeared, to reappear as suddenly when the amount of ether was slightly diminished. Accordingly the attempt to obtain the ether plateau was given up, and at 3.30 the amount of ether was left at a certain definite ratio to the air and this proportion was unchanged to the end of the experiment. At 3.52 with the threshold of the reflex at 612 units, 0.3 mgm. of strychnine was injected. One minute later the threshold was 14 units and in four minutes down to 8 units. This was only 3 units above the threshold at the beginning of the experiment three hours before, al-

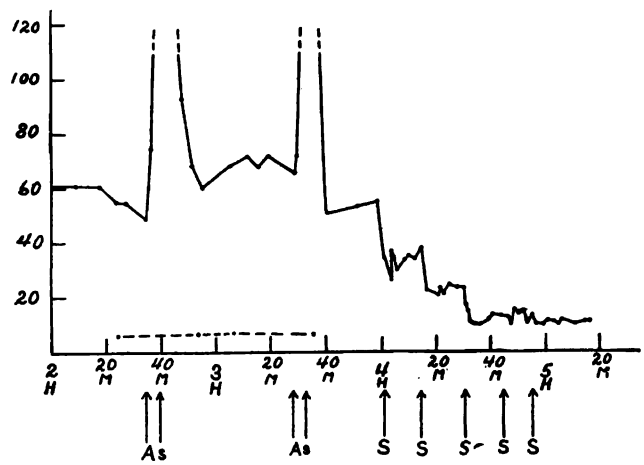

Fig. 2. Lowering by strychnine of a reflex threshold high from the effects of asphyxia. The lowering takes place in successive stages. *As*, asphyxia (artificial respiration discontinued), *S*, injections of strychnine (0.2 mgm. doses). Legend otherwise as in figure 2 (Exp. of Nov. 2, 1911).

though in the meantime it had been for two hours not lower than 480 units. Further doses of strychinne failed to affect the threshold more than slightly although convulsions were produced. In another experiment of the same kind there was a drop in consequence of injections of strychnine but this was followed by a rise as the ether made itself effective against the strychnine. A second dose of strychnine again resulted in a drop and this was repeated a third time.

The threshold can be lowered when it is high in consequence of influences other than drugs. Figure 2 is the plot of an experi-

ment in which the reflex threshold was high following asphyxia. Five successive doses of strychnine were given. Each of the first three doses forced the threshold to a point lower than it had held preceding the dose. After the fourth and fifth doses, however, although the threshold was lowered, it never went below the minimum point reached after the third injection. A similar lowering by stages has been observed when strychnine has followed ether.

Assuming that strychnine is the strongest "excitant" which could have been used, this series of experiments indicates that the flexion reflex is frequently in the highest state of irritability of which the mechanism is capable. This is not surprising. Flexion of the leg is a protective reflex in frequent use. To be

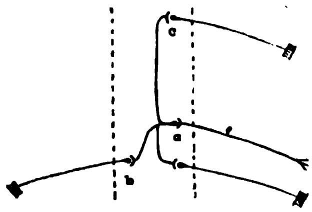

Fig. 3. Explanation in text.

most serviceable to the organism it must be set on a "hair-trigger." Here if anywhere amongst spinal reflexes we might expect to find a threshold at its lowest possible level.

Figure 3 is a modification of Sherrington's[9] diagram of neurones and synapses concerned in the production of typical spinal reflexes. Suppose (a) to represent the synapse of highest threshold in the flexion reflex arc, (b) the corresponding synapse for crossed extension, and (c) that for extension of the fore-limb. If we assign to (a) a resistance of 1, then according to my determinations (b) will have a value of 1.8 to 107, dropping to 1 or nearly so after strychnine. Synapse (c) will have a resistance infinitely high in the undrugged animal but after strychnine its resistance, like that of (b), will become approximately 1.

[9] Sherrington: The integrative action of the nervous system, New York, 1906, p. 156.

There is nothing in this series of experiments to prove that (b) and (c) may not acquire even less resistance than (a). When, in the experiment of January 17 (p. 176), at 1.10, flexion, crossed-extension and extension of fore-limb all respond to a stimulus of 2.4 Z units and not to a less stimulus it may mean merely that a flexion synapse having a resistance of 2.4, as (a), must be crossed before crossed-extension and fore-limb movement appear. But synapses (b) and (c) may conceivably have a still lower threshold.

The effects on the nervous system of strychnine and of asphyxia in its early stages are closely similar. "Increased reflex irritability" has frequently been reported in consequence of lack of oxygen. I[10] found no clear evidence of a lowered reflex threshold in asphxia although the usual convulsions, superficially resembling those of strychnine, were always observed. Perhaps, then, in asphyxia the increased reflex irritability is due not so much to the lowering of reflex thresholds toward entering stimuli as to the lowering of thresholds of many or all reflexes toward stimuli which have already entered the cord.

SUMMARY

In the spinal cat thresholds of the flexion reflex are frequently present which are not lowered by strychnine even when administered in doses causing convulsions.

Thresholds of the flexion reflex more commonly, however, are lowered by strychnine, the drop varying from 8 to 56 per cent of the original height of threshold.

The threshold of the crossed extension reflex, normally from 2 to 100 times as high as the flexion threshold, is lowered nearly or quite to the flexion threshold by strychnine. The threshold for reflex extension of the fore-limb, a movement inelicitable in the undrugged animal, is similarly lowered.

A flexion threshold which is abnormally high following administration of ether, or in consequence of asphyxia, is lowered by strychnine to approximately the normal level. The lowering may take place in stages following successive doses of the drug.

[10] E. L. Porter: This Journal, 1913, xxxi, p. 241.

CONTRIBUTIONS TO THE PHYSIOLOGY OF THE STOMACH

XX. The Contractions of the Rabbit's Stomach During Hunger

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From the Hull Physiological Laboratory of the University of Chicago

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The work of Carlson[1] on man and dogs has confirmed the conclusion of Cannon and Washburn[2] that the sensation of hunger is intimately associated with contractions of the empty stomach. Contrary to the common statement that the empty stomach is quiet and at rest, it can be easily shown that the stomach is most active when empty. But since the stomach of herbivorous animals, and especially that of the ruminants, is under normal conditions never empty and these animals undoubtedly feel hunger, the question arises of how hunger is to be explained in them. At the suggestion of Professor Carlson the subject was studied in the rabbit.

Gastric fistulas were made in the rabbits by opening the abdominal cavity about one inch to the left of the mid-ventral line and as close to the costal border as possible and suturing the muscularis of the fundic portion of the stomach to the peritoneum and oblique muscles and then, the gastric mucosa to the skin. These fistulas were made rather small so that there would be little leakage of gastric juice or loss of food. At times a small rubber tube was inserted into the stomach and left there for longer or shorter times to prevent closing of the fistula. Within thirty-six hours after the operation animals are usually feeding. Such animals were kept in the laboratory for periods of two to six

[1] Carlson: This Journal, xxxi, p. 175.
[2] Cannon and Washburn: This Journal, xxix, p. 441.

weeks. Rabbits with these fistulas, if properly taken care of, are in as good condition as normal animals. In only two or three cases were gastro-intestinal troubles noted and these were in animals which had been submitted to prolonged starvation and had nasal and pulmonary infections. About eighteen rabbits were used in these tests.

Graphic records were made using the rubber balloon method described by Carlson. The manometers and writing levers were adjusted very carefully so as to show all changes of the stomach movements. Care was taken to use as low a pressure in the writing apparatus as would clearly record the movements. This was 2 to 4 cms. of chloroform. The relative sizes of the distended balloon and stomach are shown in figure

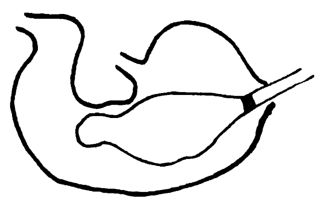

Fig. 1. The distended balloon in position in the rabbit's stomach. From a life-size tracing.

1. In this particular instance, the balloon had been inserted thirty-six hours before death and the pressure within it had not been changed, from the exterior, within that time. The space between the balloon and the stomach wall was filled with a dirty green residue.

Medium size adult rabbits were used in this set of experiments. In no case did any animal survive a period of continuous starvation of more than seven days But since they were frequently starved for several days and then fed for varying lengths of time and again starved, this short period of life on continuous starvation is not surprising. However a review of the literature shows that only large and heavy animals can stand more than two weeks deprivation of food.[3] Plenty of water was always given

[3] Luciani: Physiologie des Menschens., 1911, iv, p. 445.

the animals. But the surprising result appeared that in animals that had died of starvation, still there were always considerable amounts of residue in the stomach. In the moist condition in which this was removed from the stomach it weighed from 8 to 13 grams. Normally the moist contents of the adult rabbit's stomach weighs 90 grams or more. It therefore seemed that the rabbit was unable to completely empty its stomach.[4] Then it was noticed, that soon after being deprived of its usual food, the animal provides a substitute by eating its own excreta. This is merely confirmation of what others have noticed. Putting the animal in a wire bottom cage does not prevent this. The only practicable way found to prevent it was to enclose the animal in a cage so fitted with a lid, that the rabbit's head was held outside, its body inside the cage. No doubt such a position proved very irksome to the rabbit, but after becoming accustomed to the position the activities of the stomach were not inhibited. In an animal so fixed, the stomach, as noted by Swirski,[5] empties itself in about twenty-four hours. Nevertheless, during normal conditions of life, most certainly the rabbit's stomach is never empty.[6]

Since normally the stomach is never empty, it is to be expected that gastric digestion peristalsis will always persist. Apparently this is true up to a short time before death. But as the period of starvation is prolonged, the stomach contractions are altered. Instead of being the gentle peristalsis of normal digestion, they become relatively powerful contraction waves which rapidly follow one after the other with a tendency for each peristaltic wave to pass into a short period of tetany (fig. 2). There is no indication of rest or periodicity to these stronger contractions until a short time before death from starvation. Auer[7] has pointed out that during normal peristalsis in the rabbit, the stomach is incessantly active. During hunger the strength of these contractions is accentuated.

[4] Heidenhain: Arch. f. des. ges. Phys., xliii, Sup. heft., 1888, p. 38.
[5] Swirski: G. Arch. f. exp. Path., 1898, 41, p. 143.
[6] Heidenhain: Loc. cit.
[7] Auer: This Journal, xxiii, p. 165.

Following this period of increased activity there comes a period of depression. This is coincident with marked weakness on the part of the rabbit or even with the approach of coma. The decline in the vigor of the stomach activities comes on gradually. The contractions become weaker, of shorter duration, and alternating with short periods of rest (fig. 2). In the last stages of starvation there may occur prolonged contractions or periods of tetany lasting from two to three minutes. This confirms the observations of Patterson[8] on dogs, that the hunger contractions persist nearly up to the time of death.

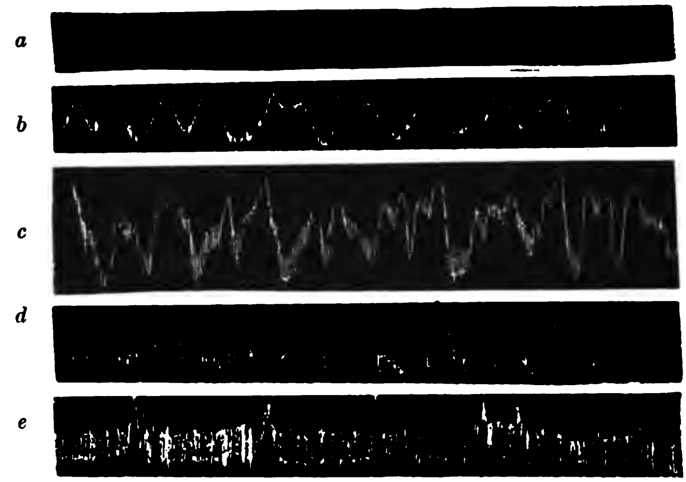

Fig. 2. Contractions of the rabbit's stomach. *a*, normal digestive peristalsis; *b*, after 24 hours starvation; *c*, after 75 hours starvation; *d*, after 92 hours starvation; *e*, after 110 hours starvation. The animal died a few hours after this tracing (*e*) was taken.

There are also differences between the normal and the hungry rabbit in the ease with which the stomach contractions are inhibited (fig. 3). This may not occur until the third or fourth day of starvation, unless precautions are taken to exclude the animal from its excreta. Water, 10 per cent alcohol, sugar solutions, 0.2 per cent–0.4 per cent hydrochloric acid, and fruit juices inhibit or weaken the contractions in the hungry animal. This was deter-

[8] Patterson: This Journal, xxxiii, p. 423.

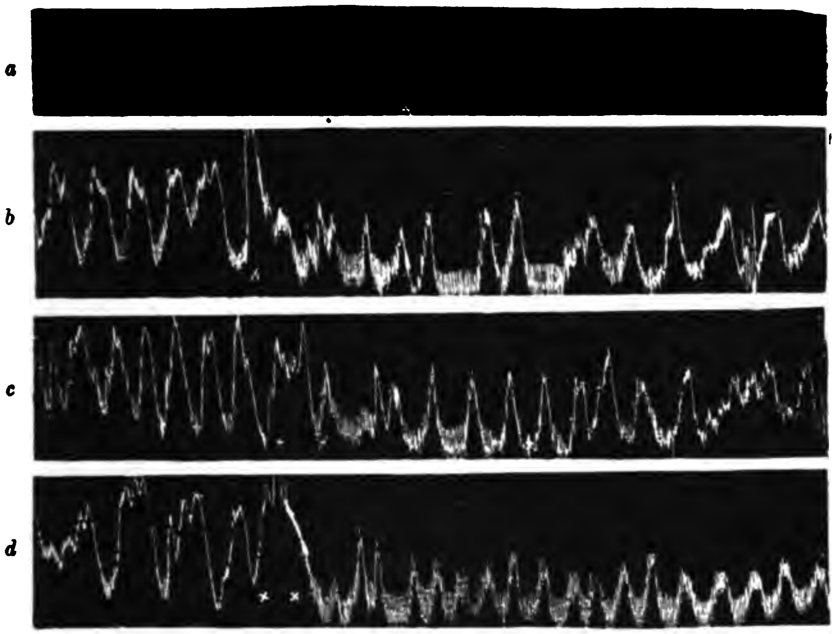

Fig. 3. *a*, 10 cc. of 0.25 per cent hydrochloric acid put into the stomach during normal digestion peristalsis; *b*, 10 cc. of 0.25 per cent hydrochloric acid put into the stomach of a hungry rabbit; *c*, 10 cc. of water put into the stomach of a hungry rabbit; *d*, 10 cc. of 10 per cent alcohol put into the stomach of a hungry rabbit. Note the inhibitory effect of these solutions on the hunger movements.

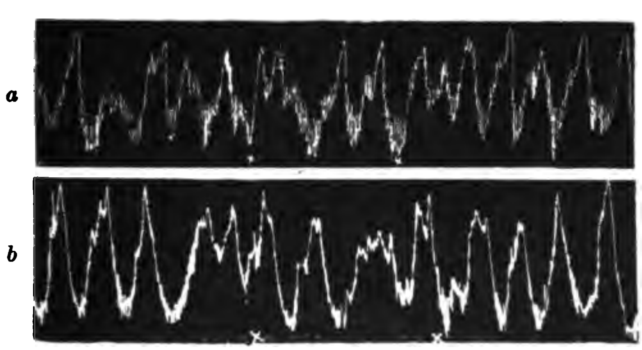

Fig. 4. Hunger contractions of a rabbit on fourth day of starvation. *a*, at X-X chewing carrot moistened with 0.4 per cent hydrochloric acid; *b*, at X-X chewing sweet carrot. No inhibition.

mined either by swallowing (water) or by introducing these solutions into the stomach through a small rubber tube passing through the fistula. In these tests especial care was taken not to excite the rabbit.

The sight, smell, taste, or chewing without swallowing, of such foods as cherries, carrots, apples and carrot leaves moistened with sugar, acid, or quinine do not inhibit the stomach

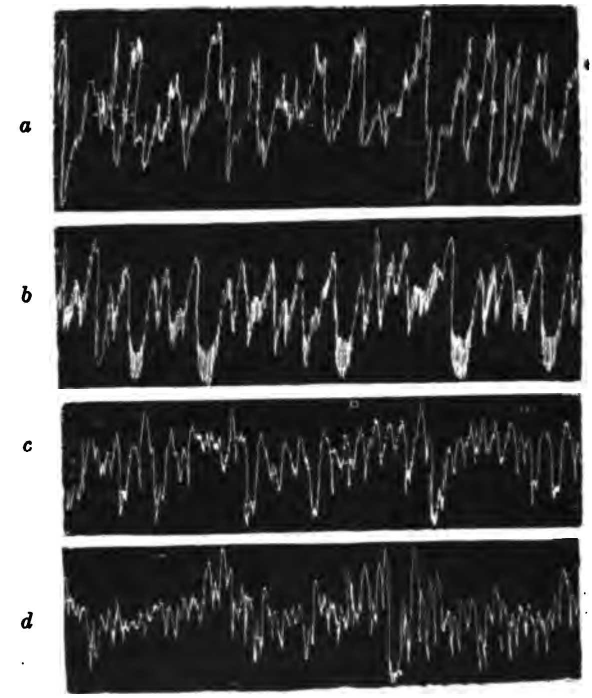

Fig. 5. Hunger contractions of a rabbit which was excluded from its feces. *a, b, c, d,* respectively contractions after 12, 24, 48 and 52 hours of starvation.

contractions (fig. 4). The relative difficulty of inhibiting the stomach contractions even when the animal is undoubtedly hungry, is in striking contrast to the ease with which this occurs in man or in dogs.[9]

Excitement not only inhibits these hunger contractions, if such they may be called, but causes a marked lowering of the

[9] Carlson: This Journal, xxxii, p. 389.

gastric tonus. In fact tonus variations were frequently seen during hunger but not commonly during normal digestion peristalsis. May not the increasing muscle tone of the stomach, as starvation is prolonged, play a part in causing the sensation of hunger? Certainly a state of greater contraction of the stomach on the substances remaining within it, as starvation is prolonged, is the first apparent change (fig. 6). Rabbits will show signs of hunger, such as restlessness, gnawing dry wood and eating cotton, before the character of the movements as recorded by this method shows any striking change from the normal digestion peristalsis. The increased activity of the stomach that later appears is no doubt also accompanied by psychic

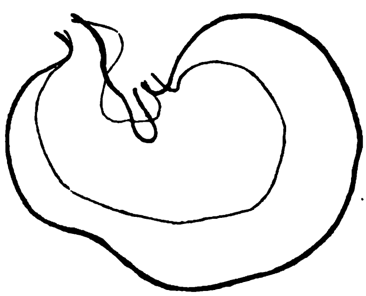

Fig. 6. Superimposed lifesize tracings of the stomachs of two adult rabbits; the larger represents the stomach of a rabbit killed after abundant eating; the smaller the stomach of a rabbit which died of starvation, but had been permitted to eat its own feces.

changes. The appearance of gastric tetany if the animal is still able to move about, is marked by restlessness.

In order to determine whether or not the stomach of the rabbit when it contains no food is quiescent, the animal was so caged as to be excluded from its feces. The only difference observed between an animal so caged and one free to move about, was that the increased vigor of the stomach contractions comes on sooner in the former. In about twelve hours after feeding the contractions become very much stronger than normal digestion peristalsis and this activity both of contractions and tonus variations persists after the stomach has emptied itself of food (fig. 5). What such an animal must feel can only be imagined

by one who has felt the periods of hunger tetany in his own stomach. The inhibitory reflexes are similar to those of the normally hungry rabbit.

SUMMARY

During starvation of the rabbit the stomach is never empty unless precautions are taken to prevent the animal from eating its feces. In the latter case the stomach of the adult rabbit empties itself in about twenty-four hours.

During starvation of the rabbit, the stomach activities are augmented and the reflexes acting on the gastric motor mechanism are different from those of normal digestion peristalsis. But there are no sharp dividing lines between the normal peristalsic contractions occurring during digestion and those occurring during hunger. *It would thus seem that in the rabbit at least, the gastric hunger contractions are intensified digestive peristalses.*

The *empty* stomach of the rabbit shows very strong and rapidly repeated contractions and marked tonus variations.

In the hungry rabbit there is a state of increased tonus in the stomach musculature. Tonus changes are more marked and occur more frequently during hunger than during normal digestion peristalsis.

The contractions of the stomach occurring during hunger in the rabbit are not inhibited by the sight, smell, taste or chewing of food.

The contractions occurring during hunger are inhibited or weakened by the introduction into the stomach of small quantities of water, weak acids, alcohol or sugar solutions. Normal peristalsis is not inhibited by the same amounts of these solutions.

Excitement (central nervous influences) inhibits the stomach contractions and causes a lowering of the tonus of the stomach musculature.

The relation of the hunger contractions to normal peristalsis is being studied further in man and dogs as well as in rabbits.

My hearty thanks are due Professor Carlson for his help and suggestions; to the laboratories of Baylor University for material; and to Drs. Aynesworth and Dudgeon for aid in the operations.

CONTRIBUTIONS TO THE PHYSIOLOGY OF THE STOMACH

XIX. Reflexes from the Intestinal Mucosa to the Stomach

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From the Hull Physiological Laboratory of the University of Chicago

Received for publication November 16, 1914

The tonus and the contractions of the empty stomach (man and dog) are temporarily inhibited by stimulation of nerves in the mouth, in the oesophagus, and in the gastric mucosa itself.[1] Can the tonus and hunger contractions of the empty stomach be influenced reflexly by stimulation of the intestinal mucosa? The answer to this question might explain the diminution or abolition of hunger by the introduction of chyme into the intestine.[2] If such reflex relations exist, it is obvious that the intestinal mucosa must be an important factor in the control of the gastric tonus and hunger mechanism.

Boldireff[3] reports that acids in the intestine inhibit the periodic activity of the empty stomach. The inhibition was not obtained by water or alkaline solutions. In fact Boldireff appears to imply that the periodic contractions of the empty stomach may be initiated by the introduction of a solution of 0.3 per cent Na_2CO_3 into the intestine. He therefore concludes that the reflex inhibition is due to an acid stimulation of nerves in the intestinal mucosa.

EXPERIMENTAL METHOD

In this work we used 24 young female dogs. Intestinal fistulæ were made by Abbe's lateral anastomosis in the first loop of the small intestine below the pancreas, the cephalad end

[1] Carlson: This Journal, 1913, xxxi, p. 212; xxxii, p. 245 and p. 389.

[2] Busch: Arch f. Pathol. Anat. u. Physiol., 1858, xiv, p. 140.

[3] Boldireff: Zentralbl. f. Physiol., 1904, xviii, p. 989.

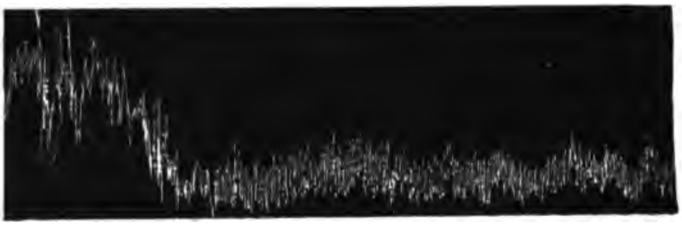
being sutured into the abdominal wall and left open to the exterior. The gastric fistulæ were made after recovery from the first operation.

In another group of dogs a Tiery fistula was made but no gastric fistula, the recording apparatus being introduced into the stomach through the oesophagus.

A

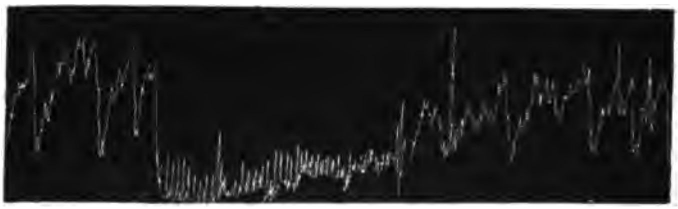
a

B



a

C



a

Fig. 1. Tracing showing tonus and hunger contractions of the empty stomach of dogs. A, 10 cc. of gastric juice introduced into small intestine at *a*. B, 10 cc., 0.5 HCl introduced into small intestine at *a*. C, 10 cc. 1 per cent Na_2CO_3 introduced into small intestine at *a*. Showing reflex inhibition of the tonus and the hunger contractions of the empty stomach by chemical stimulation of intestinal mucosa.

In a third group the gastric fistula was made near the pyloric end of the stomach. Through this fistula a small stomach tube was passed through the pylorus into the small intestine for varying distances. This tube was kept in the gut throughout the experiment for the introduction of the liquids into the in-

testine. The recording balloon was passed into the stomach either through the gastric fistula or through the oesophagus.

In the last group of dogs the vagi and splanchnic nerves were cut, and after recovery from the operation, gastric fistulæ were established in the antrum pylori. In all tests on this group the fluids were introduced into the intestine by means of a tube passed through the pylorus, and the stomach balloon was passed down through the oesophagus.

The following solutions were introduced into the intestine in 10 cc. quantities, in most cases at body temperature:

1. Normal gastric juice (dog, man).
2. 10 per cent Witte's peptone in 0.2 per cent HCl.
3. Pepsin in 0.2 per cent HCl.
4. Hydrochloric acid (0.1 per cent to 0.5 per cent).
5. Saturated H_2CO_3 solution.
6. Sodium carbonate (0.2 per cent to 1 per cent).
7. Glucose (neutral solution).
8. Neutral olive oil.
9. Fresh milk.
10. Water.
11. Mechanical stimulation of the intestinal mucosa (glass rod or rubber tube).

RESULTS

When the vagi and the splanchnic nerves are intact *all mechanical and chemical stimulations of the intestinal mucosa cause inhibition of the gastric tonus and hunger contractions.* The effect of a purely mechanical stimulation (rubbing the mucosa with a glass rod or rubber tube) is the most transitory. In general pure gastric juice and the 0.5 per cent HCl cause the longest inhibition. The acid peptone solution followed these closely. The weaker acids produced inhibition of less duration. Saturated carbonic acid solution did not give quite so distinct an inhibition as the other acids. Inhibition with pure gastric juice and the acid-peptone mixture varied in duration from three to twenty minutes, depending apparently largely on the condition of the animal at the time. The sodium carbonate solution caused

inhibition of less duration than acid mixtures but of longer duration than the water or the neutral mixtures in general. However, the longest inhibition obtained in any one experiment was produced by 10 cc. of milk in the gut. In this case the inhibition lasted thirty minutes. Ordinarily, neutral solutions produced a longer inhibition than the mechanical stimulation by moving the soft rubber tube or the stomach tube in the intestinal fistula.

In the animals with the vagi and splanchnic nerves severed the above substances still caused reflex inhibition of the empty

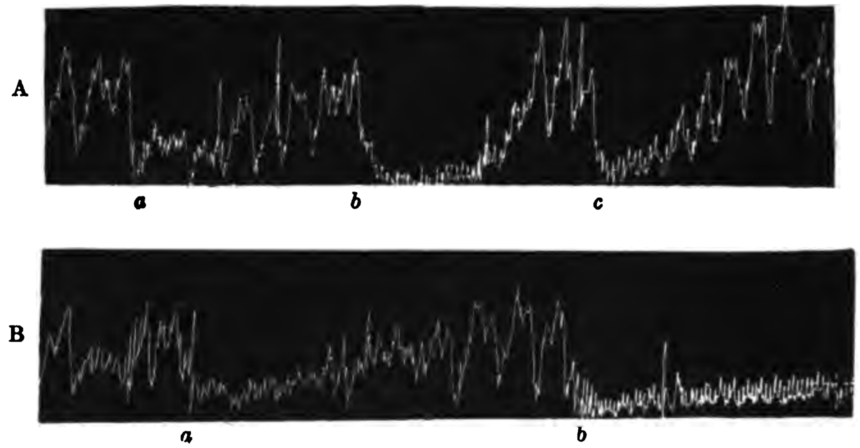


Fig. 2. Tracings from the empty stomach of dogs. A, *a*, mechanical stimulation of intestinal mucosa (gently moving a rubber tube in the lumen); *b*, 10 cc. water introduced into the intestine; *c*, 10 cc. of 0.3 per cent Na_2CO_3 introduced into the intestines. B, *a*, 10 cc. of 10 per cent peptone in 0.2 per cent HCl introduced into intestine; *b*, 10 cc. fresh milk introduced into the intestine. Showing temporary inhibition of tonus and hunger contractions of the empty stomach by mechanical and chemical stimulation of the intestinal mucosa.

stomach from the intestinal mucosa, but the latent period of the inhibition was greatly prolonged, the degree of the inhibition less, and the duration of it much shorter than in the normal animals.

It is therefore clear that this inhibition of the tonus and contractions of the empty stomach by chemical and mechanical stimulation of the intestinal mucosa involves both long or cen-

tral and short or local reflex paths, a situation similar to that found in the gastric mucosa itself.[4]

The precise rôle of this reflex in the control of the gastric hunger mechanism in the normal animal must be determined by further investigation. It is probably a factor in the diminution or absence of hunger in cases of enteritis, intestinal obstruction, and constipation.

CONCLUSIONS

(*a*) Gastric juice, chyme, acids, alkalies, water, milk and oil introduced into the small intestine inhibit gastric hunger contractions and gastric tonus for varying periods.

(*b*) This inhibition of these gastric hunger contractions and tonus is due partly to mechanical, partly to chemical stimulation of the intestinal mucosa. The chemical stimulation produces the greatest effect.

(*c*) This inhibition of gastric hunger contractions takes place apparently primarily by the "long" or central reflex path, but "short" or local reflex paths in Auerbach's plexus are also involved.

[4] Carlson: This Journal, 1913, xxxi, p. 212; xxxii, p. 245 and p. 389.

CONTRIBUTIONS FROM THE ZOÖLOGICAL LABORATORY OF THE MUSEUM OF COMPARATIVE ZOÖLOGY AT HARVARD COLLEGE. No: 258

A NOTE ON THE PHYSIOLOGY OF THE CUVIERIAN ORGANS OF HOLOTHURIA CAPTIVA LUDW.[1]

W. J. CROZIER

Received for publication November 18, 1914

CONTENTS

I. Concerning the function and mode of action of the Cuvierian organs of holothurians there is available very little experimental evidence.[2] The following observations upon the physiology of these structures in one of the Bermudan species of Holothuria may therefore be of interest.

Holothuria captiva does not bury itself in mud or sand, but is to be found (during the day-time at least) only on the under surface of slabs of stone, in places where numbers of such stones occur piled together just under low-water level.[3] It clings to the rock surfaces with such tenacity that frequently numbers of pedicels must be torn before the animal can be removed. During the process of collection there almost invariably occurs the expulsion of some of the Cuvierian organs, which in this species

[1] Contributions from the Bermuda Biological Station for Research. No. 37.

[2] For an account of the views of varius writers see Minchin (1892) and Mines (1912).

[3] Such localities were: Hungry Bay, near the entrance; Bluck's Point; and the old break-water near Walsingham, Castle Harbor. Previous papers (Crozier, 1914 a [?] and 1914 b) contain data on the behavior of these animals. The present account is based upon the experiments during the summers of 1913 and 1914, at the Bermuda Biological Station.

are very abundant (cf. Clark, 1901, p. 342). I have never seen a case in which the tubes were in an extruded condition when the rocks bearing H. captiva were turned over under water, though in all several hundred animals were observed in their natural position. I can therefore offer no suggestion as to their rôle in the normal economy of the animal.

II. It has generally been stated that holothurians possessing Cuvierian organs expel them when the animal is "irritated;" presumably mechanical stimulation of an intense character is meant. H. captiva was observed to discharge its tubes when the back or side was stimulated by repeated gentle touches with a blunt instrument, or when the papillae or tentacles were pinched. The brim surrounding the dorsally situated anus became at the same time elevated into a funnel-shaped tube, the opening of which was, in a general way, directed toward the irritated area. The number of organs discharged varied with the intensity and duration of stimulation. The individual threads were frequently as much as ten times the length of the animal producing them. Usually, but not always, one end of the Cuvierian organ remained attached to the cloaca. The general appearance and behavior of the threads followed very closely the account given by Minchin (1892) of the discharged Cuvierian organs of H. nigra.

The application of solutions of a variety of "irritating" substances to the surface of H. captiva did not lead to the discharge of the organs. Half-cubic centimeter volumes of inorganic acids ($\frac{M}{100}$), phenol ($\frac{M}{100}$), turpentine, essential oils, etc., were used. To such solutions the animal reacts vigorously, but not by the expulsion of Cuvierian organs.

A gradual increase in the temperature of the seawater, up to 40°C., did not induce the discharge of the tubes. I have previously noted, in H. surinamensis, the absence of a temperature sense (Crozier 1914a [?]).

Immersion in diluted seawater or in rain water did not lead to Cuvierian organ discharge.

I have elsewhere shown (Crozier, 1914 b) that light has a toxic action on these animals. Holothurians exposed to the

direct rays of the sun sometimes discharged a few Cuvierian organs, but after being in bright sunlight for a short time (about 15 minutes) they passed into a moribund condition, in which no tubes were expelled in response to mechanical stimulation. When kept in laboratory aquaria shielded from the light, the partial stagnation of the water was accompanied by the discharge of some of the tubes long before auto-evisceration made its appearance.

It would therefore seem that the normal stimulus for the discharge of these organs is of a tactile or mechanical nature, since it is only in response to such stimuli that the tubes are discharged promptly and elongated to any extent.

III. Mines (1912) found that the discharge of the Cuvierian organs of H. nigra was accompanied by an increase of internal pressure comparable to that which he found sufficient to produce the extension of the isolated tubes, and hence concludes that the tubes are extended by water which is forced into them, in accordance with the view of Hérouard (Delage et Hérouard, 1903). If Holothuria captiva be plunged into water at temperatures between 40°C. and 60°C., the animal contracts somewhat and several Cuvierian organs are usually expelled, though they are coagulated before they are extended beyond a centimeter or so. The internal body pressure may also be artifically raised by pressing on the holothurian's back, in which case the tubes also make their appearance. If the pressure is gentle, they are forced out and break off as small cigar-shaped bodies tapering at both ends, about 8 to 10 mm. long and 0.2 mm. in diameter (fig. 1). By pressing on the animal suddenly and with considerable force the tubes may be caused to be shot out in typical fashion. When placed in rain-water at 36°C., H. captiva dies in less than ten minutes; pressure caused the expulsion of Cuvierian organs from animals killed in this way, but they were never completely extended.

There are a number of facts which indicate that an increase in internal pressure is not the only, nor even the principal factor producing the discharge and elongation of the tubes. When

H. captiva undergoes auto-evisceration,[4] the rupture of the cloacal wall is accompanied by a considerable increase of internal pressure, but it is only rarely that any Cuvierian organs are even partially discharged during this process. Moreover, and this is a factor which has apparently been overlooked, there proceeds from the anus of an animal under experimental pressure, and also in the normal discharge of the organs, a fairly strong current derived from water previously held in the respiratory trees. The direction of this current was observed and its force estimated in water colored with carmine particles. It was clearly evident that the current served to direct and elongate the Cuvierian organs. By this means the organs are generally directed away from the animal discharging them, which is thus prevented from entangling itself. If the unextended organs secured by gentle pressure on a holothurian were taken up in a wide-mouthed pipette and ejected therefrom under the water, they became greatly elongated. A stream of water directed upon an unextended tube lying in a glass dish also produced this elongation, especially when one end of the tube had become attached by its sticky secretion; the latter condition corresponds almost exactly to the state of affairs during the normal discharge of the organs.

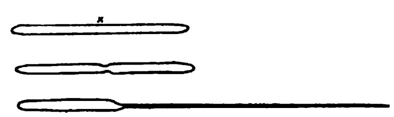


Fig. 1. Showing the reaction of a single Cuvierian organ—obtained by pressing on a Holothuria captiva—to tactile stimulation at the point x. The time occupied by the reaction was in this case 3 seconds. Magnified 2 diameters.

The cloacal stream has also another effect. As is noted by several writers (see Mines, 1912), the Cuvierian organs possess an intrinsic elongating mechanism, consisting of circular muscle

[4] Even when kept in well aerated seawater aquaria protected from the light, holothurians will eviscerate after about four days. This does not happen, however, until all ingested material has been removed from the gut by defecation. It is therefore possible that one form of the stimulus to evisceration originates in the gut, and it may be suggested that possibly the stimulus results from the autolysis of the intestine, due to the accumulation of digestive enzymes, which under normal conditions are continually removed, since the gut is always full.

fibers and some longitudinal ones. The structure of the tubes of H. captiva agrees very well with Ludwig's account of these organs in H. poli (Ludwig, 1892, p. 173). The short tubes above described as being ejected under gentle pressure may be kept alive for some time in seawater. If they are touched, as with the tip of a fine glass rod, they constrict at about the middle point and from this level proceed to elongate in both directions. Sometimes only the proximal half became constricted and elongated, the distal half—corresponding to the "head" of the organ as described by Minchin (1892) and Russo (1899)—retaining its original form. They also reacted locally by constrictions. Inasmuch as the tubes, as normally discharged, travel faster during part of their course than does the cloacal stream, the water current probably stimulates their extension by the self-contained mechanism. These holothurians when pressed upon sometimes emitted tubes as those above described, which were swirled about by the cloacal stream and stimulated to extension; whereas other tubes of the same sort upon which the cloacal stream did not impinge remained unextended. I therefore believe that the increased body pressure observed by Mines is primarily related to the production of the cloacal stream, and only secondarily implicated in the discharge and elongation of the tubes.

There are, then, at least three factors involved in the discharge and extension of the Cuvierian organs; the internal body pressure, the mechanical and stimulating action of the cloacal current, and the intrinsic extending mechanism of the tubes.

The nervous path of the stimuli leading to Cuvierian organ expulsion is not known. With the object of discovering whether the oral nerve ring took part in the matter, I experimented upon animals in which the anterior end had been cut off. The tubes were somet'mes ejected by individuals so prepared, but never in a fully extended condition. The result is not decisive, since animals with the anterior end intact frequently presented the same condition after they had been in the laboratory for some time. With such animals, in which the general level of tone is very low, the unextended tubes could be obtained by pressure very much more easily than with the specimens in good

condition. This points to the conclusion that the normal release of the Cuvierian organs includes the action of the nervous system, though perhaps in an indirect way.

IV. When the tubes are in the discharged condition they are very sticky. In tubes ejected in water above 40°C. the stickiness is not so evident, even when the tubes are drawn out. Neither are they sticky when extruded into rain water. The adhesive property is somewhat developed when the rain water has been made faintly alkaline. Hence the stickiness depends upon the presence of a substance coagulable by heat, which acquires its adhesiveness through the action of the alkalinity and salts of sea water.

The coelomic fluid of Holothuria is practically sea water. Why, then, do not the tubes stick together as they lie in close contact in the body cavity? It has been generally observed that the tubes are adhesive only when extended. As a matter of fact, the stickiness increases in the slightly extended tubes after they have been for some minutes in seawater. But it would seem that in the usual rapid elongation of the organs the adhesive material is probably squeezed out of gland cells by the contraction of the wall of the tube— a conclusion which is favored by the structure of the organ.

V. SUMMARY

1. The Cuvierian organs of Holothuria captiva are expelled in response to mechanical stimulation, but not to various forms of chemical irritation.

2. The mechanism of their discharge comprises an intrinsic extending apparatus acting together with the stimulating action and directive influence of the cloacal current. The Cuvierian organs are not expelled by the forcing into them of water from the cloaca.

3. The adhesiveness of the discharged Cuvierian organs depends upon the presence of a heat-coagulable substance, which is altered in such a way as to become sticky when it is acted upon by the alkalinity and salts of sea water.

REFERENCES

CLARK, H. L. 1901. Bermudan echinoderms. Proc. Bost. Soc. Nat. Hist., vol. 29, no. 16, pp. 339–345.

CROZIER, W. J. 1914 a (?). The sensory reactions of Holothuria surinamensis Ludw. Zool. Jahrb., Abt. f. Physiol. (in press).

CROZIER, W. J. 1914 b. The orientation of a holothurian by light. Amer. Jour. Physiol., vol. 36, pp. 1–13, 6 figs.

DELAGE, Y., et HÉROUARD, E. 1903. Les échinodermes. Traité de Zool. concrète, T. 3, x + 495 p., 565 fig., 53 pls.

LUDWIG, H. 1892. Die Seewalzen. Bronn's Thier-Reich, Bd. 2, Abt. 3, Buch I, vi + 460 p., 25 figs., 17 pls.

MINCHIN, E. A. 1892. Notes on the Cuvierian organs of Holothuria nigra. Ann. Mag. Nat. Hist., Ser. 6, vol. 10, pp. 273–284, pl. 17.

MINES, G. R. 1912. Note on the mechanism of discharge of the Cuvierian organs of Holothuria nigra. Quart. Jour. Micr. Sci., vol. 57, pp. 301–304, pl. 26.

RUSSO, A. 1899. Sur valore morfologico e funzionale degli organi di Cuvier delle Oloturie. Monit. Zool. Ital., anno 10, pp. 225–228, 2 fig.

THE VASO-MOTOR NERVES OF THE DUODENUM

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The arteria gastro-duodenalis which forms the continuation of the hepatic artery, gives rise to the arteria gastro-epiploica dextra and the arteria pancreatico-duodenalis. The former supplies the uppermost portion of the duodenum and adjoining pyloric end of the stomach, while the latter is directed towards the head of the pancreas, where it anastomoses with the arteria pancreatico-duodenalis inferior. During its course along the border of the middle segment of the duodenum this blood vessel gives off a number of branches which ascend upon both walls of this part of the intestine.

The nerve fibres originally embraced in the hepatic plexus, reach these parts by following the highways of the arteries. The plexus gastro-duodenalis divides into two networks at the point of bifurcation of the artery. A part of it then invades the pyloric region and another the pancreatic tissue, but besides, a number of filaments become separated from the main plexus and accompany the arterial twigs to the central segment of the duodenum.

In accordance with its blood and nerve supply the duodenum of the dog may be divided into three parts, namely, into an upper, a middle and a lower segment. While this peculiar anatomical arrangement has enabled me to prove the existence of vasomotor nerves in the region of the pylorus[1] as well as in the lowermost portion of the duodenum,[2] it has been quite impossible so far to furnish similar proof for the central extent of this part of the intestine. This failure is to be attributed to the difficulties

[1] Burton-Opitz: Pflüger's Archiv, cxlvi, 1912, 344.
[2] Burton-Opitz: Pflüger's Archiv, cxxiv, 1908, 469.

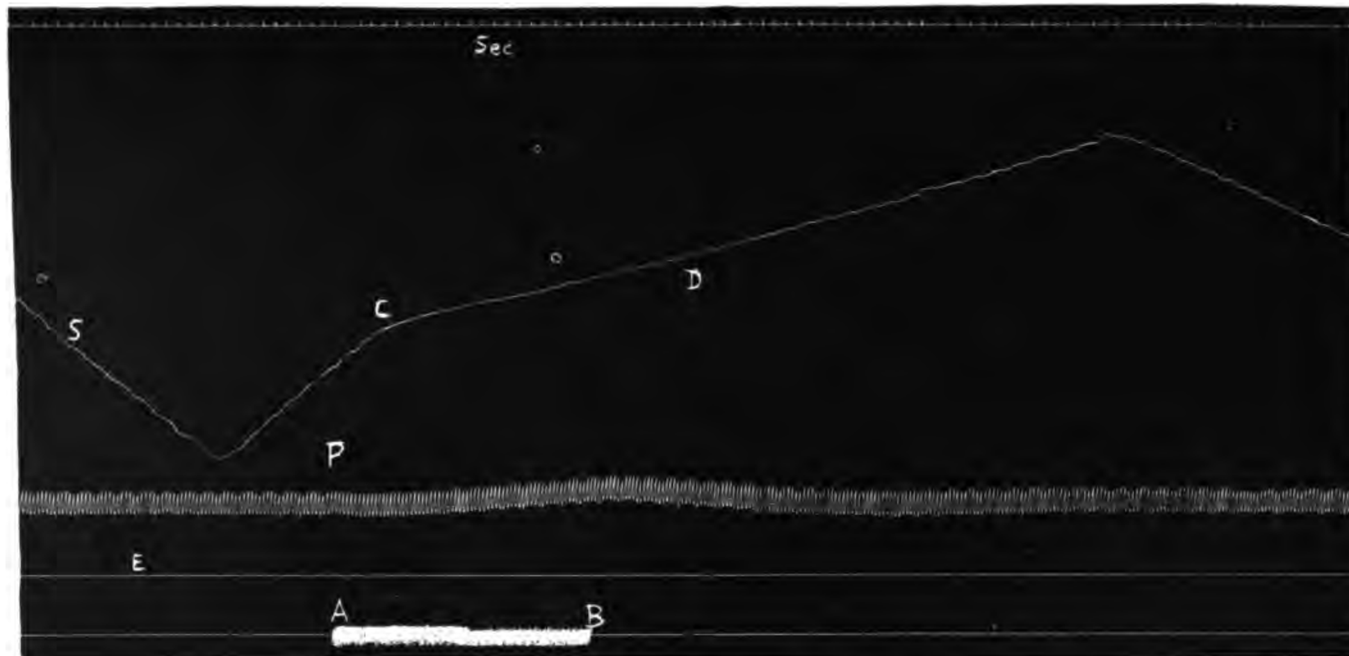
encountered when a separation between the pancreatic tissue and the duodenum is attempted. To be sure, the blood supply of a large part of the pancreas can be determined satisfactorily, because this organ can be isolated from the intestine by simply ligating the duodenal branches of the pancreatic artery. In spite of the fact that the area of contact between the organs in question is not equally large in all animals; the reverse procedure meets with many obstacles, because the removal of the pancreatic tissue is generally attended by complete obliteration of many of the delicate duodenal blood vessels.

The animal from which the present data have been derived, presented rather favorable conditions in so far as the pancreatic artery pursued a very superficial course, so that its duodenal ramifications could be freed without serious injury to them and the accompanying nerve fibres. Having ligated the arteria gastro-epiploica dextra, the vascular field was restricted still further by obstructing all pancreatic branches. A stromuhr[3] was then inserted in the arteria gastro-duodenalis. The central cannula of this instrument gave attachment to the connecting tube of a membrane manometer for the determination of the pressure existing in this blood vessel. The distal end of the divided plexus gastro-duodenalis was placed in shielded electrodes at a point opposite the stromuhr.

This procedure enabled me to measure the quantity of blood distributed to the middle portion of the duodenum, while stimulation of the aforesaid plexus was resorted to at intervals. But, as it is extremely probable that the ligation of the different branches of the pancreatic artery markedly enhanced the flow through the duodenal channels, the quantitative data here given should be regarded merely as differential values.

In view of the present experimental arrangement, it must be clear that any variation in the blood supply of this part coincident with the excitation of this plexus, must be produced by active changes in the size of the duodenal blood-bed. To begin with the stromuhr registered a flow of 0.32 cc. in a second which, upon

[3] I employed the recording stromuhr described by me in Pflüger's Archiv, cxxi, 1908, 150.



Blood Supply of Duodenum

stimulation of the gastro-duodenal plexus with a moderate current, slowly decreased to 0.13 cc. in a second. The blood pressure which previously amounted to only 68.5 mm. Hg, rose gradually to 79.0 mm. Hg. Normal vascular conditions were again established soon after the cessation of the stimulation.

The curve reproduced in the accompanying plate shows the record of the stromuhr (*S*) in exact relation with the record of the membrane manometer (*P*) above the common abscissa *E*. The stimulation of the plexus gastro-duodenalis took place between points *A* and *B*. It is evident that the blood flow is considerably lessened during the seventh phase of the stromuhr, the decrease becoming well marked within a few seconds after the beginning of the stimulation. Between points *C* and *D* the flow attains its lowest value, while the arterial pressure rises to its highest level The excitation having been discontinued at *B* the flow and the pressure show a gradual return to normal at *D*.

These results which unmistakably point towards an active peripheral obstruction of the blood flow, were obtained several times in the course of this experiment. As the vascular field had been restricted in this animal to the central duodenum, it must be concluded that the blood vessels of this organ are innervated by nerves which ascend from the coeliac ganglion by way of the plexus gastro-duodenalis and plexus pancreatico-duodenalis.

THE BODY SURFACE OF FLOUNDERS AND ITS RELATION TO THE GASEOUS METABOLISM

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It is an obvious fact that the surface of every organ bears an important relation to its function, and nature, by employing various means, has given to small masses surfaces a thousand times or more greater than would follow from their geometrical form. One thinks, naturally, of the lung, occupying a relatively small volume of the chest, but which has an exposure to the air representing an area of several thousand square meters, thanks to innumerable branches of the bronchi ending in minute alveoli. The blood which receives oxygen in the lung and distributes it to the various tissues is assisted in this office to an amazing degree by the great number of minute elements carrying oxygen, the red blood corpuscles, whose composite surface is very great as compared to their total mass. Such examples could be easily multiplied but even those already referred to suffice to point out the physiological significance of surfaces.

The outside surface of the entire organism, on the contrary, tends to approach as nearly as possible the geometrical minimum, apparently to reduce the area of contact with the surrounding medium and thus prevent unnecessary loss of heat. The exposure must, therefore, be comparable for all organisms in so far as it represents the smallest surface compatible with their mass. It is hereby assumed that the specific gravity is the same, which may be the case under similar physiological conditions. It must likewise be specified that this law of the minimum exposure may be counteracted by some special provisions for preventing the loss of heat, such as thickness of the skin or the nature of its covering.

The measurement of surface has been rarely undertaken because of its many difficulties. In the case of the higher mammals the prevailing methods are so cumbersome as to discourage their extensive use. As the consideration of surface relations, however, has attained much importance in the physiology of metabolism, a careful investigation seemed to me highly desirable. For this purpose I availed myself of the flounder, whose flat body can be easily and accurately measured. The method was extremely simple. The flounders were killed by pithing and weighed after the last traces of adherent water were removed. They were then laid flat on a stiff sheet of paper with the fins fully spread out, and an outline of the body was traced with a sharp pencil. The fins were then trimmed off close to the body, and an outline was again made of the finless animal. The weight was again determined, and a third outline made of the upper part of the specimen, which generally has a slight curvature. The area of this part of the flounder could be traced easily by rotating the flounder on its long axis. The surface was gotten from these three outlines which were measured by means of a planimeter. The maximum length, width and thickness of each specimen were likewise noted.

If the specific gravity of the body were one—in other words, if its mass and volume were equal, the surface could be expressed by the simple formula $S = \sqrt[3]{W^2}$ where W is the weight. As a matter of fact the surface is invariably greater than this, and under normal conditions is expressed by the formula $S = K\sqrt[3]{W^2}$, where S is the surface and K a constant factor. In metabolism studies this factor K determined for some particular animal has been applied to a wide range of physiological conditions. It will be shown in this report that results obtained in this way are highly unreliable.

By actually measuring the total surface of a large number of flounders, I computed the value of the factor K from the above formula. A glance at the subjoined table 1 will convince one that a very definite relation exists between surface and weight. The value of K ranges from 12.00 to 14.87 and from 7.82 to 9.59 for flounders with and without fins respectively. An examination

of the column of constants reveals that as the weight of the flounders increases the value of K tends to be smaller Averaging all the recorded values of K for flounders less than 10 cm. long, we find that it is 13.48 and 8.67 respectively; for flounders of over 10 cm. in length the average value of K is 13.37 and 8.25 respectively. For the entire lot of about forty specimens the average is 13.44 (or 8.5 exclusive of the fins).

The slightly higher constant for small flounders may perhaps suggest that they have a proportionately larger surface, but I am strongly convinced that it is due to the fact that they are frequently more poorly nourished than their more successful competitors for food, the larger flounders. At any rate, fasting, as will be shown later, invariably causes a very considerable increase in the value of K.

An examination of the data pertaining to the other measurements of each flounder shows some very interesting things. It will be observed that the width and especially the thickness change very slowly in comparison with the weight, but the length (from the tip of the snout to the base of the caudal fin) varies directly with it. Thus, with the maximum thickness increasing threefold—0.65 to 1.95 cm.—the body weight changed from 5.7 gram (average) to 148.2 grams, or 26 times. For a similar increase in width—from 1.8 to 5.4 cm.—the body weight changed from 1.45 grams to 43.5 grams, or 30 times. In both instances, however, the length changed from 6.85 to 19.80 cm. and from 4.6 to 13.0 cm. respectively; i.e., in either case practically in the same ratio as the weights (1 ÷ 3). The close relation between the weight and length is of much importance, as it affords a means of estimating the weight when the length is known, and vice versa.

The weight of the fins forms a comparatively small portion of the total mass of the fish, as will be seen by the figures of the third column. It will be noticed also that as the flounder grows bigger and heavier the fins become relatively lighter. The surface of the fins, however, represents 30–40 per cent of the total body surface. It is, therefore, possible to cause a considerable reduction of surface without appreciably affecting the weight

of the fish by cutting off the fins. If the rate of metabolism depended upon the surface, as some believe, it would be natural to expect a drop in the oxygen consumption concomitant with the removal of the fins. Experiments bearing on this question are described below.

A series of experiments were performed with three small flounders for the purpose of finding out the rate of oxygen consumption with a normal and reduced body surface. The results recorded in the first part of the table below show a considerable range of variation depending upon the state of nutrition and, especially, the behavior. After one week's experimentation all the fins were removed in two specimens, while in the third—the smallest— only the left fin was removed. The animals were apparently little discomforted by this operation, swam about at times quite actively and ate the food. Several experiments were then performed under conditions similar to those of the previous set. It will be noticed from the results of the second part of the table that the oxygen consumption per gram-hour is not different from what it had been before the fins were removed (except when the animals have been particularly restless) and the surface thereby diminished.

As the second part of the experiment in the foregoing series was performed several days after the operation a few other experiments were made to determine the more immediate effect of removing the fins. A flounder weighing 7.65 grams was used. In a preliminary experiment it was found that its oxygen consumption per gram-hour was 0.179 cc. At the close of the experiment all fins were removed and about an hour later a new experiment performed under precisely the same conditions. The oxygen consumption per gram-hour was 0.172 cc. this time. This flounder died the next day.

In the case of another flounder weighing 9.58 grams the oxygen consumption before and after the operation was 0.177 cc. and 0.154 cc. per gram-hour respectively. In either case the diminution in weight occasioned by the removal of the fins was insignificant (2.5–3.1 per cent), but the surface was reduced by fully one-third.

TABLE 1

WEIGHT IN GRAMS	WEIGHT IN GRAMS (WITHOUT FINS)	WEIGHT OF FINS IN PER CENT OF THE TOTAL WEIGHT	BODY MEASUREMENTS IN CM.				$S=K\sqrt[3]{W^2}$		SURFACE OF FINS IN PER CENT OF TOTAL SURFACE	VALUE OF K FOR TOTAL SURFACE	VALUE OF K FOR BODY SURFACE EXCLUSIVE OF FINS
			Total length	Same without caudal fin	Width	Thickness	Total body surface in sq. cm.	Same, exclusive of the fins			
0.695	0.655	5.8	4.3	3.7	1.3		10.54	7.22	33.2	13.45	9.58
0.755	0.710	6.0	4.5	3.7	1.4		12.33	7.33	40.5	14.87	9.21
0.845	0.790	6.5	4.6	3.9	1.4		12.96	8.20	36.8	14.56	9.59
1.045	0.975	6.7	4.9	4.0	1.6		14.05	9.21	34.5	13.64	9.36
1.300	1.220	6.2	5.2	4.3	1.6		14.30	9.62	32.7	12.00	8.42
1.445	1.340	7.3	5.5	4.6	1.8		17.57	11.19	36.4	13.75	9.21
1.730	1.640	5.2	5.8	4.8	1.9		19.28	12.68	34.2	13.40	9.10
1.840	1.740	5.4	5.7	4.6	1.85		19.38	11.88	38.7	12.90	8.21
1.870	1.820	?	5.8	4.8	2.0		19.56	12.96	?	12.88	8.69
2.340	2.230	4.5	6.3	5.2	?.1		22.70	14.76	35.0	12.88	8.64
2.610	2.475	5.2	6.5	5.45	2.15		24.94	15.31	38.7	13.16	8.37
2.630	2.480	5.7	6.45	5.25	2.10	0.50	26.00	15.78	39.3	13.64	8.62
3.075	2.880	6.3	6.6	5.5	2.10		26.76	16.24	39.3	12.65	8.02
3.090	2.980	3.6	6.7	5.6	2.30		26.92	18.00	33.1	12.69	8.69
3.490	3.260	6.6	6.9	5.7	2.35		29.63	18.75	36.7	12.88	8.53
4.035	3.860	4.3	7.5	6.2	2.35	0.55	35.58	21.30	40.1	14.04	8.66
4.300	4.120	4.2	7.5	6.2	2.40	0.65	34.76	21.30	38.7	13.15	8.29
4.690	4.480	4.5	7.8	6.55	2.55		41.04	24.24	40.9	14.65	8.92
5.750	5.520	4.0	8.3	6.85	2.60	0.65	42.11	26.11	38.0	13.12	8.36
5.800	5.560	4.1	8.0	6.75	2.70	0.65	40.88	25.20	38.3	12.67	8.03
6.110	5.870	4.0	8.5	7.00	2.70	0.65	44.13	26.71	39.5	13.20	8.21
7.740	7.350	5.0	9.1	7.40	3.05	0.70	53.94	31.68	41.3	13.79	8.38
8.280	7.940	4.1	9.5	7.80	3.15	0.75	54.30	34.56	36.4	13.27	8.68
11.180	10.67	4.6	10.6	8.50	3.40	0.75	73.23	41.85	42.9	14.65	8.63
13.13	12.60	4.0	10.5	8.60	3.70	0.85	75.90	46.25	39.0	13.64	8.55
15.35	14.88	3.1	11.4	9.40	3.65	0.95	80.80	48.32	40.2	13.08	8.00
15.50	14.91	3.8	11.55	9.35	3.65	0.95	85.33	50.09	41.3	13.72	8.28
22.80*	22.00	3.5	12.95	10.55	4.15	1.05	103.57	61.37	40.8	12.88	7.82
25.57	24.78	3.1	14.00	11.50	4.50	1.05	119.22	71.96	39.7	13.73	8.47
26.50	25.65	3.2	13.90	11.30	4.50	1.05	124.08	72.97	41.2	13.95	8.39
28.45	27.47	3.5	14.10	11.60	4.50	1.10	124.76	74.58	40.2	13.38	8.19
42.64	40.73	4.5	15.70	12.80	5.10	1.15	155.70	95.09	38.9	12.75	8.03
43.50	41.95	3.6	15.90	13.00	5.40	1.25	165.15	100.39	39.2	13.35	8.32
49.56	47.78	3.6	16.80	13.60	5.70	1.30	186.11	109.19	41.3	13.79	8.29
52.85	50.83	3.8	16.90	13.90	5.50	1.35	183.06	109.20	40.3	13.00	7.96
90.39	86.53	4.3	20.70	16.70	6.20	1.65	277.54	157.72	42.5	13.78	8.06
90.87	87.44	3.8	20.60	16.60	6.20	1.65	265.82	156.10	41.3	13.15	8.00
148.20	142.87	3.6	24.60	19.80	7.90	1.95	364.93	221.83	39.2	12.32	8.12

* Ripe female.

TABLE 2

DATE	BODY WEIGHT OF FLOUNDER			OXYGEN USED PER GRAM-HOUR BY FLOUNDER			REMARKS
	A	B	C	A	B	C	
	gm.	gm.	gm.	cc.	cc.	cc.	
VII. 4	6.99			0.182			24 hours after feeding.
VII. 5	6.84	4.15	1.62	0.162	0.186	0.128	24 hours fasting (A, 48 hours). A and B somewhat restless.
VII. 6	7.59	4.12	1.68	0.240	0.137	0.154	14 hours after feeding. A very restless.
VII. 7	7.52	3.97	1.60	0.160	0.160	0.157	Not fed. B somewhat restless.
VII. 8	7.34	3.98	1.66	0.202	0.143	0.181	Only C took food. A restless.
VII. 10	7.89	4.08	1.72	0.198	0.192	0.241	Fed liberally since July 8.
VII. 13	7.46	3.76	1.63	0.205	0.252	0.193	24 hours after feeding A and B very restless.
VII. 14	7.27	3.75	1.58	0.253	0.264	0.191	A and B very restless! Not fed yesterday.
VII. 18	7.20	3.44	1.72	0.171	0.174	0.221	Fed freely several days. Last feeding about 24 hours before experiment.
VII. 19	7.31	3.34	1.66	0.192	0.185	0.209	Were not fed yesterday. A slightly restless.
VII. 23	7.32	3.45	1.86	0.192	0.196	0.268	Fed regularly till yesterday. Fins have regenerated appreciably.

Still another experiment was performed with a large flounder weighing 49.58 grams. Before the operation its oxygen consumption per gram-hour was 0.101 cc., but after the operation 0.116 cc. This increase is rather noteworthy, especially since owing to the loss of the fins the flounder kept exceedingly quiet during the second part of the experiment. I am strongly inclined to believe that the strong contraction of the muscles of the body which immediately resulted from the removal of the fins is responsible for this increased demand for oxygen. During the following couple of days the oxygen consumption dropped very considerably, but the animal was sickly and soon died. More experiments with large flounders will, therefore, be necessary to determine the effect of the removal of the fins.

Under Rubner's influence the general belief prevails that the gaseous metabolism is proportional to the body surface, and that

per unit of area it is the same for all animals. The theoretical basis for this generalization is the simple physical principle of radiation of heat which is proportional to the exposed surface. Since the metabolic processes are associated with the production of heat, the latter depending largely upon the rate of its dissipation to the outside environment, the metabolic processes must bear a direct relation to the surface. The figures generally given in support of this generalization cannot, without a stretch of the imagination, form a solid foundation for it. Pütter,[1] indeed, is very emphatic in pointing out the great variability of these figures and in his contention that the body surface has no relation to metabolic processes. But the lack of actual measurements of surface, above everything else, deprives Rubner's theory of its scientific value.

In the table that follows data which have been gathered at different times are brought together. These throw an entirely new light on this problem. The weight, surface and oxygen requirements have been actually measured. In a few instances the surface was not measured at the same time when the weight was determined. In these cases both the actually found surface and the theoretically expected surface are recorded. The difference between these two is of little significance.

Weight of flounder in grams	0.70	1.63	2.83	4.02	4.04	5.29	6.99	7.65	9.58	24.0	44.5
Oxygen consumption per hour in cc	0.170	0.314	0.523	0.662	0.762	1.038	1.270	1.370	1.690	4.08	6.25
Oxygen consumption per gram-hour in cc.	0.241	0.193	0.185	0.155	0.189	0.196	0.182	0.179	0.177	0.170	0.140
Surface of flounder in sq. cm	11.41 (10.49)	18.42	26.21	33.63	34.76 (33.74)	38.82 (40.38)	48.62	51.64	59.99	109.00	162.18
Oxygen consumption per sq. cm.-hour in cc.	0.015 (0.017)	0.017	0.020	0.020	0.022 (0.023	0.027 (0.026)	0.026	0.026	0.028	0.037	0.038

It must be inferred from these results that, while per unit of body weight the oxygen consumption diminishes as the size

[1] Pütter, August, 1911. Active Oberfläche und Organfunktion, Zeitschr. f. allg. Physiol., 12, 125–214.

of the flounder increases, the oxygen consumption per unit of body surface increases very regularly. In other words, the larger the flounder the larger its oxygen requirements per square cm. This very obviously results from the direct dependence of the metabolic exchange upon the mass of the organism which grows more rapidly than the surface.

The value of K is nearly constant so long as the physiological conditions remain the same. We are, therefore, quite unjustified in computing the surface of a fasting organism, for instance, from the formula $S = K\sqrt[3]{W^2}$ as is the common practice, using the value K established for a normal individual. There are two reasons for this: first, the body surface does not diminish in the same ratio as the body weight; second, the specific gravity is lower than normal owing to the greater content of water in the fasting organism. Measurements of surface reveal that these two factors conspire to raise the value of K. In the following table are given the results obtained from the study of several flounders which have been deprived of food for 5 to 28 days. At the close of the fasting experiment both the weight and surface of each specimen were carefully measured. Comparing these data with those recorded in the first table it is seen that the area of the organism has changed only to a very slight degree. It will also be of interest to observe that the relative weight and the proportion of the area covered by the fins at the end of a long fasting period remains practically the same as before fasting.

DAYS OF FASTING	INITIAL WEIGHT	FINAL WEIGHT		RELATIVE WEIGHT OF FINS	BODY DIMENSIONS				INITIAL SURFACE (estimated)			FINAL SURFACE		RELATIVE SURFACE OF FINS	VALUE OF K	
		Total	Without fins		Total length	Length (to caudal fin)	Width	Thickness	Value of K (computed)	Surface	Surface (K=13.44)	Total	Same without fins		Total surface	Same exclusive of fins
	grams			*per cent*	*cm.*									*per cent*		
22	0.705	0.415	0.375	9.6	4.25	3.50	1.35	0.20	14.19	11.41	10.32	11.24	6.88	38.8	20 21	13.23
15	3.30	2.165	2.06	4.9	6.9	5.7	2.15		12.86	29.17	29.48	28.60	17.46	39.0	17.09	10.78
28	4.04	2.69	2.55	5.2					13.50	34.76	33.74	34.25	19.99	41.6	17.71	10.58
28	5.29	3.47	3.32	4.3	7.75	6.40	2.65	0.45	13.60	38.82	40.38	38.25	23.07	39.7	16.72	10.37
5	44.5	41.96	40.36	3.8	16.1	13.6	5.4	1.20	12.40	162.18	167.02	159.78	101.1	36.8	13.22	8.59

The value of K, which for normal flounders has been found to be 13.44, varies from 17.09 to 20.21 and from 10.37 to 13.23 for flounders with and without the fins respectively. (The case of the large flounder where the fast was too short to induce a noticeable change is neglected.) The initial surface is estimated by two methods. First, the value of K is computed from the formula $S = \sqrt[3]{W_1^2}$, where W_1 – the initial weight – is substituted for W – the final weight. From the average of these constants the deviation from the normal is determined, which in this case is about 2.5 per cent, and a corresponding correction is introduced in the total surface. The second method consists in estimating the initial surface with the aid of the average constant (K = 13.44) which has been established for normal flounders.

If one compares the oxygen consumption of the first four flounders at the beginning and end of the fast the interesting thing appears that per square cm. the oxygen decreases in every instance to from one-third to two-thirds of the original amount. If, however, the final body surface—which in this experiment has been actually measured—were estimated from the final weight with the aid of the constant 13.44, as is generally practiced, it would be found that at times the oxygen consumption per square cm. remains unchanged. This is shown in the table below:

INITIAL SURFACE IN SQ. CM.	OXYGEN IN CC. PER SQ. CM. AND HOUR	FINAL SURFACE (MEASURED)	OXYGEN IN CC. PER SQ. CM. AND HOUR	FINAL SURFACE (COMPUTED WITH FACTOR 13.44)	OXYGEN IN CC. PER SQ. CM. AND HOUR
11.41	0.015	11.24	0.010	7.47	0.015
29.17	0.026	28.60	0.019	22.44	0.024
34.76	0.022	34.25	0.011	31.67	0.012
38.82	0.027	38.25	0.008	30.84	0.011

It is obvious, therefore, that a scientifically unqualified trust in mere computation is probably responsible for the broad generalization according to which the metabolic processes are the same when referred to the unit of surface. The coincidence of the data for the oxygen consumption per square cm. in the

first two cases may serve as a warning against conclusions which have been drawn without any foundation or reference to the facts.

The following conclusions may be drawn from the above investigation. The surface of flounders can be computed by the ordinary formula $S = K\sqrt[3]{W^2}$, whereby the average value of the constant factor K for *normal* flounders has been found to be 13.44. This coincides very closely with the value which has been found for higher organisms.

The metabolic processes of the flounder bear direct relation neither to the weight nor surface of the flounder, the oxygen consumption per gram-hour diminishing but increasing per square centimeter-hour as the flounders become larger.

Removal of the fins, causing a reduction of surface of over 30 per cent without materially changing the mass of the flounders, does not affect the oxygen consumption, provided no disturbing factors are present. Under these conditions the oxygen consumption is proportional to the mass.

The value of K changes according to the physiological state of the organism. For fasting flounders, owing to the fact that the surface does not diminish in the same ratio as the mass, and the specific gravity of the body also decreases, the value of K is considerably higher than normal and must be determined in each individual case.

THE INFLUENCE OF PREGNANCY ON THE HYPERGLYCEMIA OF PANCREATIC DIABETES

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It has been shown that late pregnancy prevents the characteristic onset of severe diabetes following complete pancreatectomy. To account for this, the suggestion was made that in pregnancy near term the internal secretion from the fetal pancreas passes into the maternal blood in sufficient amount to maintain the normal carbohydrtate metabolism.[1]

In the previous work the presence or absence of diabetes was determined by urine examination alone. There is a mere possibility that late pregnancy in dogs may have some injurious action on the kidneys so as to diminish or prevent the excretion of sugar, in which case we might have the hyperglycemia of diabetes without glycosuria. This possibility has now been tested on four pregnant dogs near term, and we desire to place on record a summary of our results in the present report.

EXPERIMENTAL PROCEDURE

1. Blood Sugar

The blood sugar was determined by the Rona-Michaelis method.

2. Drawing Blood

The blood (about 5 cc.) was drawn either from the saphenous vein by a hypodermic needle, or from the tail. If sufficient care is taken there is no pain or struggling to cause temporary hyperglycemia, even when no local anaesthesia is employed.

[1] Carlson and Drennan: This Journal, 1911, xxviii, p. 391; Carlson, Orr and Jones: Jour. of Biol. Chem., 1914, xviii, p. 19.

In every case a sample of normal blood was drawn before the pancreatectomy and at varying intervals after the operation until the pups were born. The animal was watched as closely as possible night and day so that samples of blood could be drawn at the onset of labor and also after parturition.

RESULTS

We succeeded in getting only ten suitable dogs, as the pregnant bitches near term are not readily obtained. Complete pancreatectomy was made in all of these dogs, but six yielded no results, as abortion occurred within seven to twenty-one hours after the operation.

Pregnant Bitch I

The normal blood sugar content in this animal before pancreatectomy was 0.08 per cent. The following eight hours after the operation there was a marked hyperglycemia. This is probably post-anaesthetic and post-operative hyperglycemia. Twelve hours later the blood sugar returned to its normal level and remained there for the subsequent thirty-one hours. The dog remained in good spirits. When labor set in there was an abrupt

TABLE I

Pregnant Bitch I. Complete pancreatectomy May 28, 1914. Recovery good. No infection

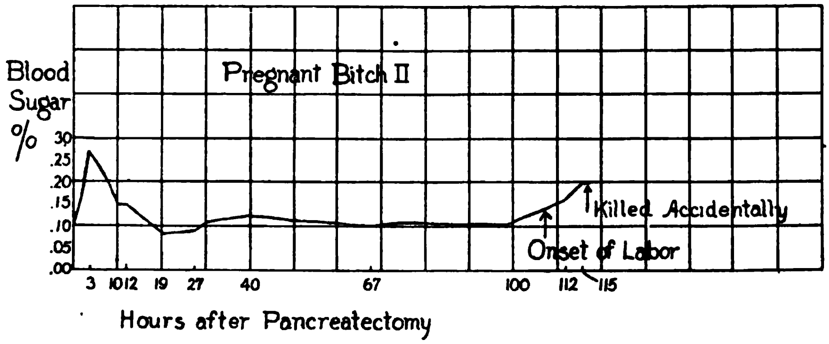
HOUR SAMPLE TAKEN	BLOOD SUGAR
	per cent
Normal	0.08
3 hours after pancreatectomy	0.111
8½ hours after pancreatectomy	0.174
12 hours after pancreatectomy	0.090
15 hours after pancreatectomy	0.090
19 hours after pancreatectomy	0.090
24 hours after pancreatectomy	0.070
31[1] hours after pancreatectomy	0.113
40[2] hours after pancreatectomy	0.200

[1] Dog in labor, one pup born.
[2] Five pups born, experiment discontinued.

rise in the blood sugar to the high level (0.20 per cent) present in experimental pancreatic diabetes and in the typical clinical condition of "severe diabetes" in man. The fact that the blood sugar rose and remained at this level indicated that the pancreatectomy was complete. This was verified at necropsy.

Pregnant Bitch II

After the operation this dog was given a daily diet of 150 cc. goat's milk, 100 grams of lean meat, and plenty of water. Following the initial post-operative hyperglycemia this dog ran a typical normal blood sugar curve up to noon of the fifth day



(112 hours) when the dog went into labor. A blood sample taken then showed 0.15 per cent sugar. Another sample taken three hours later showed 0.20 per cent. As the animal was weak and the uterine contractions apparently very feeble, we feared that she would not be capable of delivering the pups, and decided to perform a Caesarean section, but, unfortunately, while preparing for this operation, the bitch was accidentally killed. At autopsy one pup was found engaged in the vagina and nine others were found in utero, of these five were still living. The pups were practically at full term. The duodenum was carefully examined but no trace of pancreatic tissue was found.

TABLE II

Pregnant Bitch II. Complete pancreatectomy June 19, 1914. Recovery good. No infection

HOUR SAMPLE TAKEN	BLOOD SUGAR
	per cent
Normal	0.100
3 hours after pancreatectomy	0.270
10 hours after pancreatectomy	0.150
12 hours after pancreatectomy	0.147
19 hours after pancreatectomy	0.087
27 hours after pancreatectomy	0.105
40 hours after pancreatectomy	0.123
67 hours after pancreatectomy	0.101
100 hours after pancreatectomy	0.119
112 hours[1] after pancreatectomy	0.150
115 hours[2] after pancreatectomy	0.200

[1] Dog in labor.
[2] Dog accidentally killed; 10 pups in utero.

Pregnant Bitch III

This dog retained her fetuses thirty-one hours, when abortion occurred and three premature pups were delivered. The sample of blood taken just before abortion revealed a blood sugar content of 0.23 per cent, showing that the break between the uterus and the pups had already occurred. The fact that the blood sugar

TABLE III

Pregnant Bitch III. Pancreatectomy July 14, 1914. No infection

HOUR SAMPLE TAKEN	BLOOD SUGAR
	per cent
Normal	0.097
3 hours after pancreatectomy	0.160
9 hours after pancreatectomy	0.160
12 hours after pancreatectomy	0.155
17 hours after pancreatectomy	0.160
25 hours after pancreatectomy	0.159
31[1] hours after pancreatectomy	0.230
43 hours after pancreatectomy	0.400

[1] Three pups born.

never completely returned to its normal level would seem to be due to lack of sufficient pancreatic tissue in the three pups, especially as these pups were premature (about three weeks of term). At subsequent autopsy no pancreatic tissue was found in the mother.

Pregnant Bitch IV

This dog retained the fetuses two full days, and except for a temporary post-operative rise the blood sugar was slightly above normal throughout. There was no sugar in the urine.

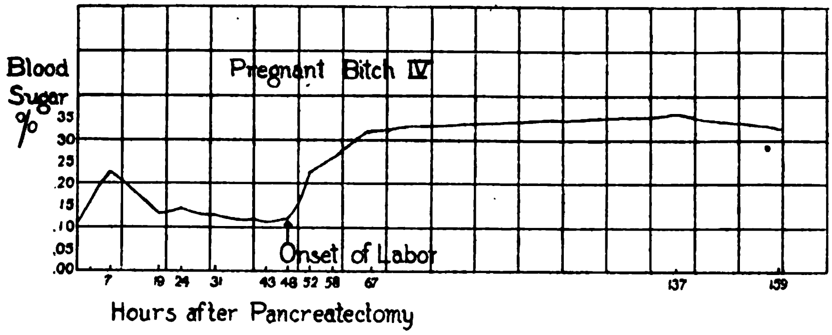


TABLE IV

Pregnant Bitch IV. Pancreatectomy July 31, 1914. Good recovery. No infection

HOUR SAMPLE TAKEN	BLOOD SUGAR
	per cent
Normal	0.110
7 hours after pancreatectomy	0.200
19 hours after pancreatectomy	0.130
24 hours after pancreatectomy	0.140
31 hours after pancreatectomy	0.119
43 hours after pancreatectomy	0.114
48 hours[1] after pancreatectomy	0.113
52 hours[2] after pancreatectomy	0.220
58 hours after pancreatectomy	0.260
67 hours after pancreatectomy	0.320
137 hours after pancreatectomy	0.360
159 hours after pancreatectomy	0.330

[1] Two pups born.
[2] Six pups born.

Two pups were born at the end of the forty-eight hours, but the blood sugar was not affected in the least, as there were still six fetuses in the uterus. When these fetuses severed their connection with the maternal organism four hours later, the blood sugar rose immediately. No pancreatic tissue was found at autopsy.

SUMMARY

Total extirpation of the pancreas in non-pregnant dogs results in the onset of pancreatic diabetes within seven to twelve hours. Complete pancreatectomy in pregnant bitches near term is not followed by hyperglycemia and glycosuria, etc., as long as the fetuses are alive and the placental connections are not severed. At the onset of labor the blood sugar begins to rise, so that the hyperglycemia with consequent glycosuria, characteristic of pancreatic as well as severe clinical diabetes is established on the completion of the delivery.

This absence of diabetes may be due either to a secretion from the fetal pancreas reaching the maternal blood, or to some detoxicating action on the part of the fetal pancreas. We hope to definitely prove or disprove the first possibility by transfusion experiments now in progress.

ON THE VALIDITY OF INDUCTORIUM CALIBRATIONS

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As the result of an extended study, with the aid of the string galvanometer, of induction shocks generated under various conditions Erlanger and Garrey[1] have recently pointed out certain differences in rate of growth, amplitude, duration, or contained current that may be shown by induction shocks of equivalent physiological intensity. On the basis of these observed differences they are inclined to question the validity of the systems of inductorium calibration now in vogue. Inasmuch as I have developed and used one such system[2] I desire to consider its validity in the light of their observations.

Degree of accuracy required. When an induction shock traverses a mass of living tissue the current composing the shock distributes itself among the various conducting pathways of the tissue according to their relative resistances, in obedience to the law of divided circuits. Of these various conducting pathways many doubtless are non-irritable, so that the physiological effect of the shock is exerted only by that part of it which comes into direct contact with irritable tissue. This irritable tissue, moreover, is undoubtedly in a state of unstable electrolytic equilibrium, undergoing frequent variations in conductivity, especially as the tissue becomes active in response to the stimulus of the shock itself. Furthermore, the structure of most tissues, with non-conducting lipoid membranes distributed throughout the general conducting mass, is that of a system of condensers, so that we have to reckon with the phenomenon of capacity, and varying capacity, as well as with the other variables involved. In view

[1] Erlanger and Garrey: This Journal, xxxv, 1914, 377.
[2] Martin: The measurement of induction shocks, New York, 1912.

of these facts one may reasonably anticipate that shocks which may be perfectly superimposed physically will produce different physiological effects at different times in one and the same tissue. As a matter of fact such differences of effect do occur constantly. We speak of them as due to changes of irritability in the tissue, although in reality they are doubtless often the result of changes in the distribution of the currents among the different conducting pathways, irritable and non-irritable, therein. One may well wonder, not that tissues display variations in responsiveness, but that they ever remain so constant as they frequently do.

I have only fragmentary data concerning the extent of error which may result from the sources just mentioned. On the basis of such as I have my practice has been to assume that the error may often amount to 10 per cent of the initial value of the stimulus. Since these sources of error are inevitable in all physiological employment of induction shocks I take the view with regard to the quantitative use of such shocks, held generally by workers in science, that refinement of one portion of a procedure beyond the inherent error of other portions is not only unnecessary, but is likely to convey an impression of greater accuracy than actually exists. In my own researches I do not base conclusions on observed differences amounting to less than 10 per cent unless they occur so frequently under particular conditions as to afford assurance that they are not accidental. In the development of an inductorium calibration for physiological use, therefore, I feel that as a criterion for stimuli that are to be rated as physiologically equivalent, exact physical superimposability is excessive.

In 1908 I urged the importance of the physiological criterion as the proper basis for a physiological evaluation of induction shocks.[3] That position I still hold, and the particular question I wish to consider in this paper is whether the calibration I have developed satisfies reasonably the physiological criterion. The problem presents itself in two phases: (1) does the calibration afford valid basis for comparisons of relative stimulation strengths on any one coil, and (2) can valid comparisons be made between different coils?

[3] Martin: This Journal, xxii, 1908, 116.

The calibration curve as an expression of relative stimulation values. To express relative stimulation values for the various positions of the secondary coil with respect to the primary, resort has been had, in most of the systems thus far proposed, to curves based on determinations of the relative amounts of contained current in shocks generated at the different secondary coil positions, by the break of a fixed primary current. Erlanger and Garrey object to this method (p. 442) on the strength of observations in which they showed that approximating the coils modifies so profoundly the *contour* of the shocks that physiologically equivalent fractions include different amounts of contained current. In making these observations Erlanger and Garrey desired to avoid variations in the total resistance of the secondary circuit. They therefore used a low resistance shunt (32 ohms) across the terminals of their secondary coil, and led off from this through their test muscles threshold fractions. A necessary result of this arrangement was that to get fractional shocks of threshold magnitude the secondary coil had to be brought into the immediate region of the primary. In one coil they used positions 12.2 cm. and 0 cm.; in another 11.1 cm. and 0 cm. (p. 441). I pointed out in my first presentation of a calibration scheme[4] that in inductoria with iron cores, such as Erlanger and Garrey used, the relation of contained current to physiological efficiency holds for different secondary positions only over the *outer* parts of the field, regions beyond the influence of the iron core. That this relationship does hold over the outer parts of the field I have proved by a large number of experiments in which more than a dozen different inductoria have been used. The failure of agreement of contained current with physiological efficiency in the portions of the field where the secondary coil overlaps the primary, necessitates, as I showed formerly (loc. cit.), the abandonment of the physical criterion for this part of the scale, and the substitution for it of a physiological criterion. The one I used, the v. Fleischl test, was used also by Erlanger and Garrey in their investigation, and appears to be valid for all comparisons for which *threshold* stimuli

[4] Martin: Loc. cit., 121.

can be employed. By means of this physiological test I showed that a calibration can be worked out for the part of the field where the physical criterion fails, and that the calibration so established is sufficiently accurate for the needs of physiologists.

The influence of secondary resistance on the form of the calibration curve. Erlanger and Garrey point out (p. 445) that the effect of changing the secondary resistance is to alter the configuration of an induction shock, and also to modify its stimulating effectiveness; facts which have been noted likewise by earlier observers.[5] They offer the opinion that this effect of secondary resistance on configuration, taken in connection with the influence of approximating the secondary coil to the primary, and with possible other factors, still unknown, complicates the problem of evaluating faradic stimuli beyond present possibility of solution. I am inclined to agree with them, so far as purely physical means of evaluation are concerned. Nevertheless, while they are doubtless correct (save for the inherent variations noted above, p. 223) in their statement (loc. cit., p. 447) that shocks would be equal physiologically if they could be exactly superimposed physically, Erlanger and Garrey would doubtless admit the possibility of the existence of shocks, equivalent physiologically, which could not be superimposed physically. The working physiologist is interested in the physiological equivalence of shocks. The test as to whether they are thus equivalent must be, in the last analysis, a physiological test. Granting that the effectiveness of shocks is modified by variations in secondary resistance, the problem is, can a valid method be worked out for taking this modification into account?

In attacking such a problem one must consider not only the effect upon physiological intensity of changes of resistance when all other factors remain constant, but also the effect of such changes in connection with others occurring simultaneously. An example of the latter situation cited by Erlanger and Garrey, and by them urged as evidence against the validity of inductorium calibrations, is the case in which there is approximation of the secondary coil to the primary in connection with changes in sec-

[5] For references see Martin: This Journal, xxvii, 1910, 226; and xxviii, 1911, 49.

ondary resistance (p. 447). Although, as shown above, Erlanger and Garrey were in error in their view that approximating the secondary coil to the primary invariably disturbs the relationship between physical and physiological intensity, a disturbance which actually appears only in that restricted portion of the field comprehended within the length of the primary coil, it will perhaps be instructive to consider the question they raise with the aid of the physiological criterion.

If simultaneous changes in secondary resistance and secondary coil position affect the physiological efficiencies of shocks so profoundly as Erlanger and Garrey intimate, obviously a calibration scale for the various secondary position which holds for low resistances should not hold for high ones. To test this point I have examined a number of the experiments I performed during the period 1906–1910 in connection with the development of a calibration scheme. In these experiments the threshold stimulus was determined for various secondary positions by the v. Fleischl method. Frogs' gastrocnemius muscles and frogs' sciatic nerves were stimulated. The values of the stimuli were expressed in *Z* units.[6] In my experiments the intensity of the shocks was cut down to threshold dimensions by cutting down the primary current, instead of by using a shunt as did Erlanger and Garrey.

In these experiments three different inductoria were used, all of the general type recommended by me for quantitative work,[7] having secondary coils about 13 cm. long, with approximately 10,000 windings. I have collected the data from 104 experiments. In 43 the total secondary resistance was less than 10,000 ohms, ranging as low as 3,100 ohms; in 24 the secondary resistance fell between 10,000 and 20,000 ohms; in 11 between 20,000 and 30,000 ohms; and in 26 above 30,000 ohms. I will report, first, the data covering the *outer* part of the field of the inductorium, the region where, according to my observations,[8] the physiological effects of shocks are proportional to their "contained currents." In each experiment threshold determinations were made at several secondary positions In each of eight experiments

[6] Martin: The measurement of induction shocks, 73.
[7] Martin: Ibid., 89.
[8] Martin: This Journal, xxii, 1908, 121.

only three such determinations were made in the outer part of the field, namely, beyond 12 cm. In each of the remaining 96 experiments four or more determinations were made in this region. If the calibration scale is correct, and the irritability of the experimental tissue does not vary, the values of Z for any one experiment should be constant Approximate constancy over a large series of experiments suffices to establish the correctness of the calibration. In each experiment I determined the average Z from all the values, found experimentally, beyond 12 cm. Then the percentage divergence from this average of the most divergent single reading was calculated. Comparisons of the number and extent of these percentage divergences, under different conditions as regards secondary resistance, afford a means of judging fairly whether or no changes in secondary resistance affect the validity of the portion of the calibration scale now under consideration.

In the 43 experiments with low secondary resistance (less than 10,000 ohms) 36 showed no single reading diverging from its average as much as 7 per cent; 4 showed single divergences falling between 7 and 10 per cent; in each of the remaining three there were single divergences greater than 10, but less than 20, per cent. In the 24 experiments with secondary resistance between 10,000 and 20,000 ohms 21 showed no divergences exceeding 7 per cent, and in none of the experiments was there a divergence as great as 10 per cent. In the 11 experiments with secondary resistance between 20,000 and 30,000 ohms, 9 held constant within 7 per cent, and none showed divergence amounting to 10 per cent. In the 26 experiments with secondary resistance exceeding 30,000 ohms 19 showed no divergence above 7 per cent, and only 2 of the 26 showed divergences exceeding 10 per cent. Furthermore, any distortion of the calibration scale which might be brought about by changing the secondary resistance ought to show itself by similar divergences in experiments with similar secondary resistance. As a matter of fact the divergences which occurred were scattered irregularly over the whole field, and doubtless were due, for the most part, to errors in exact establishment of the threshold.

On the basis of the facts cited thus far I would offer the following conclusion: *A calibration scale, established for the outer part of the field of a "standard" inductorium, on the basis of the "contained current" in the shocks, expresses accurately the influence, on the physiological efficiency of the shocks, of shifting the secondary coil, no matter what the resistance in the secondary circuit.*

Turn now to the situation which exists in the inner portion of the field, that portion within the influence of the iron core. Here are realized conditions which, according to Erlanger and Garrey, tend to invalidate calibration scales, namely, the possible simultaneous occurrence of two distinct influences, shifts in secondary coil position and variations in secondary resistance, either of which affects shock contour independently of the other. It is quite true that in this part of the field a calibration scale that holds for low secondary resistances will not be wholly correct for high ones. This I pointed out in 1912.[9] The problem here is strictly a practical one. Recognizing the desirability of including this portion of the field so far as possible in a quantitative scheme, can a calibration be established in which the limits of error shall not be so great as to destroy its serviceability? On the ground that a calibration which is accurate within 15 per cent is useful, if its limitations are clearly understood, I have examined the same series of experiments as was discussed above, to learn to what extent the calibration worked out by me for the inner portion of the field departs from this degree of accuracy with different secondary resistances. The calibration was established originally by observations on tissues whose secondary resistances would rarely exceed 10,000 ohms. In the experiments with secondary resistances below this figure divergences from the average value of Z exceeding 15 per cent were relatively few, numbering only 17 out of 108 readings, the latter made at various points on the scale between positions 0 cm. and 8 cm. Of these 17 marked divergences, 11 were above the average and 6 below, showing that they were accidental, rather than indicative of an error in the calibration. The experiments in which resistances greater than 10,000 ohms occurred

[9] Martin: The measurements of induction shocks, 59.

can best be considered in two divisions; first with reference to the portion of the inductorium field from the outer end of the primary coil as far in as position 6 cm.; and second with reference to the very innermost portion of the field. For the first division readings at positions 8 cm. and 6 cm. were used exclusively, since a calibration that is shown to be valid for these positions must hold for regions beyond. Of 51 readings at positions 8 cm. and 6 cm., with secondary resistances above 10,000 ohms, 7 diverged from the average more than 15 per cent. Of these 7 divergences, 6 occurred in experiments in which the secondary resistance exceeded 30,000 ohms. All 7 were below their respective averages, as were also more than 70 per cent of the minor divergences. At secondary positions 4 cm. and 0 cm. the experiments included 82 readings in which secondary resistance exceeded 10,000 ohms. Of these 82, 33 were more than 15 per cent divergent. All these large divergences were below their respective averages, as were also more than 80 per cent of the minor divergences. Of the 33 large divergences of this series, 24 were from among the 40 cases in which the secondary resistance exceeded 20,000 ohms.

A just interpretation of the above data seems to me to be that *a calibration scale established for the inner portion of the inductorium field by experiments upon tissues of low resistance holds with less and less accuracy for high secondary resistances the closer the secondary coil is approximated to the position of complete superposition, and also the greater the resistance; but that for practical purposes the calibration is sufficiently accurate over the range of resistances likely to occur in physiological work as far in as position 6 cm. in a "standard" inductorium.* In accordance with this view my practice of late in calibrating inductoria has been to carry the calibration no further toward the zero position than 6 cm. The slight resultant diminution in range of stimulating power is readily compensated for by increasing the primary current.

The relation of secondary resistance to the physiological efficiency of faradic stimuli. The discussion presented in the above paragraphs has for its sole purpose the demonstration that a *cali-*

bration applied to a "standard" inductorium, in accordance with the scheme I have developed, is valid with reference to threshold stimuli generated in any tissue regardless of the resistance of the tissue. The further question, whether, in two different tissues of the same resistance, shocks identical according to the calibration, would necessarily be physiologically equivalent, requires special consideration. In a former paper[10] I have discussed this question exhaustively, and have shown that although stimuli for individual tissues must be dealt with individually, so that comparisons of stimuli imparted to one tissue with those imparted to another cannot be made directly, with a high degree of accuracy, yet in those cases in which threshold irritabilities can be determined, allowance can be made for the factors which tend to render direct comparisons inaccurate. I see no occasion for reviewing here the method by which this is done. I wish to say only that application of the method in hundreds of experiments has been fruitful of information not likely to have been afforded by procedures in which it was not employed.

The applicability of the calibration to stimuli above the threshold. Erlanger and Garrey (loc. cit., p. 442) question the applicability of a calibration based on threshold shocks to stimuli above the threshold. It happens that the observation which occasions their question is the incomplete one previously discussed of the effect on "contained current" and physiological efficiency of approximating the coils. That the disturbance of the relationship between these factors occurs only in the inner portion of the field has been pointed out above. Significant in this connection is the fact that the disturbance of relationship in this region applies fully to *threshold* shocks, so that in a calibration established for this region according to the method I have employed the indicated physiological efficiencies will not be proportional to the contained currents. While the raising of this question is thus seen not to be necessitated by any of the reported observations of Erlanger and Garrey, the problem may well be considered briefly on its own merits. The fundamental assumption on which rests the validity of the calibration for stimuli

[10] Martin: This Journal, xxvii, 1910, 226.

above the threshold is that *so long as other factors remain constant, the effectiveness of a stimulus is directly proportional to the intensity of the primary current whose break or make generates it.* Erlanger and Garrey report no galvanometric studies of the effects on induction shocks of varying the primary current, but such evidence as is available from other sources accords completely with the assumption. One such line of evidence is derived from a study of the data obtained in the preparation of a calibration scale for an inductorium. An early step in the procedure is the determination, in the outer part of the field, of the galvanometer deflections brought about at different secondary positions by the break of a *fixed* current through the primary. If a fixed current is not actually used the results are reduced to the values they would have if the current were fixed. These deflections are plotted in the form of a curve and are considered to represent the relative physiological efficiencies of the secondary positions at which they were obtained. To prove that they do truly bear this relationship the v. Fleischl test is applied. In this test the *primary current is adjusted* until a threshold shock is found. The curve of galvanometer deflections, based on a fixed primary current, is deemed correct if the products of the deflections at the various secondary positions by the primary currents required to generate threshold shocks at the same positions give a constant. As a matter of fact constants are obtained regularly under these conditions, although the irritabilities of the test tissues vary so greatly that the primary currents required in different experiments to generate threshold shocks at particular secondary positions will scarcely be found twice alike, and may vary over an enormous range. I find, for example, among the 104 experiments cited in earlier paragraphs of this paper, primary currents, used at position 12 cm. to elicit threshold shocks, ranging from 0.0003 ampere, to 0.1 ampere. If the physiological efficiencies of shocks were not directly proportional to the intensities of the primary currents used in generating them, constant results could not be obtained under these conditions.

A second line of evidence is afforded by experiments in which threshold fractions of shocks are obtained by means of shunts in the secondary circuit. Where shunts are used thus, the primary currents required to generate shocks of threshold intensity will differ according as the resistance of the shunt varies. We have here a means, therefore, of determining directly whether changes in the primary current bring about equivalent variations in physiological effect. In the use of this test the influence of secondary resistance on physiological effectiveness must be taken into account. This can readily be done by applying the principle upon which rests my system of correcting for secondary resistance, namely, that if the values of Z obtained experimentally by eliciting equivalent shocks with various secondary resistances, are plotted against the resistances, the resulting curve is a straight line, whose formula I have determined.[11] The actual data from a test of this character are as follows: Experiment of February 5, 1909. Values of Z (primary current times "calibration number") were determined at secondary coil positions 8 cm., 12 cm., 18 cm., and 24 cm., for the threshold of a frog's sciatic-gastrocnemius preparation under three conditions of secondary resistance; (1) with the tissue only (res. 4900 ohms) in circuit with the secondary coil (res. 1400 ohms); (2) with 6000 ohms added resistance in the secondary circuit; (3) with a shunt of 2000 ohms resistance in the secondary circuit. With each of these resistances closely concordant values of Z were obtained for the four secondary positions at which tests were made. Their average values, together with the total secondary resistances, were: for (1) $Z = 2.73$, total secondary resistance = 6300 ohms; for (2) $Z = 3.45$, total secondary resistance = 10,300 ohms; for (3) $Z = 7.4$, total secondary resistance = 2800 ohms. If to the figures of (1) and (2) the Wilbur modification[12] of my equations for correcting for secondary resistance, $\beta = \frac{Z_{R}R' - Z_{R'}R}{R' - R}$, be applied, the value of β for this particular preparation can be calculated, and, in turn, from this

[11] Martin: Loc. cit., 228.

[12] Martin, Bigelow and Wilbur: This Journal, xxxiii, 1914, 416.

value and the data of (1) the theoretical value of Z for any other secondary resistance can be determined. By applying these calculations, the theoretical value of Z for a secondary resistance of 2800 ohms (the resistance of (3)) is found to be 2.10. The experimental value of Z for (3), 7.4, represents a shock that divided itself between the tissue (res. 4900 ohms) and the shunt (res. 2000 ohms). If from the equation for the flow of currents through divided circuits we compute the fractional part of Z (3) that passed through the tissue, it is found to have a value of 2.15. This agrees with the theoretical value 2.10, determined above, well within the limit of error. Repetition of this experiment with other secondary resistances and other shunt resistances have given equally concordant results. In this experiment primary currents of greater than threshold intensity were used. No impairment of the relationships expressed by the calibration was manifest. We may conclude, on the basis of these two lines of evidence, that the stimulating effectiveness of induction shocks is directly proportional to the intensity of the primary currents used in generating them. If this conclusion is correct the assumption is equally justified that the relationships expressed by an inductorium calibration for different secondary coil positions are valid for stimuli above the threshold as well as for those just at the threshold. In my own work I have used both these methods of varying stimulation strength very freely, and have encountered no conditions which would suggest that they are not perfectly interchangeable.

The applicability of the calibration to various sorts of tissues. Erlanger and Garrey suggest (p. 421) that inasmuch as the "*Nutzzeit* plays an important rôle in determining the physiological value of currents of short duration" and since the *Nutzzeit* varies in different kinds of tissue, a calibration that is valid for one kind of tissue might prove erroneous when applied to another. Here, again, we have a question whose answer must be sought in direct experimentation. I find, upon referring to the series of 104 experiments cited in earlier paragraphs, that in 47 of them frogs' muscles, *uncurarized*, in 8 frogs' muscles, *curarized*, in 47 frogs' sciatic nerves, and in the remaining 2

areas of human skin, were stimulated. The calibration applied as accurately to the experiments with any one of these tissues as to the experiments with any other. This general applicability of the calibration signifies that by means of it the relative strengths of stimuli as applied to any one class of tissues, no matter which one, can be accurately expressed. It does not show whether or not relationships can be stated accurately between stimuli applied to tissues having different *Nutzzeiten*. The chief significance of the *Nutzzeit*, to judge from the observations of Gildemeister,[13] is in situations in which it is exceptionally long. In ordinary laboratory tissues, in which the stimulus, even of a rapid induction shock, definitely outlasts the *Nutzzeit*, it is not probable that the sources of error due to variations in the *Nutzzeit* are so much greater than those mentioned above as inherent in the electrical stimulation of tissues as to require special consideration, in calibration schemes whose ideal is the maximum of serviceability, even at the sacrifice of the last degree of accuracy. In other words, the *Nutzzeit*, as a factor in comparisons of stimuli, is of importance to students of the excitation process as a physiological phenomenon, rather than to users of excitations as means of evoking other physiological activities.

Comparisons of shocks generated by different inductoria. Erlanger and Garrey emphasize the difficulty of making valid quantitative comparisons of shocks generated by different inductoria. In this they are fully justified. As Gildemeister showed,[14] and as I have also pointed out,[15] inductoria of dissimilar construction are likely to produce shocks which have very different physiological effects even though the "contained current" in the shocks compared may be exactly the same. Erlanger and Garrey show (p. 403) that these differences in physiological effect are due to differences in shock contour. I might mention incidentally that in my own comparisons of dissimilar inductoria, calibrated according to the "contained current"

[13] Gildemeister: Zeitschrift fur Biologie, lxii, 1913, 359.
[14] Gildemeister: Archiv fur die gesammte Physiologie, cxxxi, 1910, 604.
[15] Martin: This Journal, xxviii, 1911, 49.

method, I have been able repeatedly by varying the secondary resistance, a procedure which also modifies shock contour, to find a resistance at which the inductoria would give equal physiological effects for equal contained currents. One such case I have described previously.[16]

The remedy for this difficulty is obviously the one suggested by Erlanger and Garrey (p. 448) and earlier by myself,[17] namely, the adoption of a standard of inductorium construction for quantitative work. My continued experience in the calibration of inductoria serves only to emphasize in my own mind the desirability of so doing, although indicating also that a certain degree of latitude may be permitted without completely vitiating the quantitative method. I take this occasion to urge as I have done formerly,[18] the Kronecker specifications as suitable for inductoria which are to be used quantitatively.

A specific objection urged against my calibration scheme by Erlanger and Garrey, applicable only so far as comparisons between different inductoria are concerned, is that the theory upon which I have proceeded demands that the time to maximum of break shocks should be constant, whereas they find, experimentally, that it varies in different inductoria (loc. cit., p. 404). They cite only two experiments in which this variation appears (tables 5 and 6, p. 403). They used different gap intervals in these two experiments, although on the previous page they assert that "the form of a shock is profoundly influenced by certain conditions such as length of the gap. . . . ." Of the three coils they studied, one, coil 1, has relatively such coarse wire in the secondary, and appears, from study of all their data to be so inefficient, as to suggest the desirability of rejecting it as an unsuitable instrument for quantitative work. Their other two coils, numbered 2 and 3, are very dissimilar structurally yet the time to maximum of break shocks generated by them agrees within 7–20 per cent.

[16] Martin: Ibid., 54 (table III).
[17] Martin: Ibid., 53.
[18] Martin: The measurement of induction shocks, 89.

In my opinion, one may safely assume that in well constructed coils, agreeing reasonably with the Kronecker standard, the time to maximum of break shocks will not vary so considerably as to vitiate the calibration. My feeling in this regard is strengthened by the very considerable experience I have had in comparing inductoria, in connection with the preparation of calibrations. I have not encountered such failures of physiological effect to agree with expectation as would occur frequently if the times to maximum of shocks from various coils differed so widely as to vitiate my method.

The knife-blade key. Erlanger and Garrey point out (p. 385) that the mercury key I have described[19] often fails to give clean cut breaks. This defect was pointed out to me also by Professor Dodge of Wesleyan University, who was using the key at the Nutrition Laboratory of the Carnegie Institution of Washington. Forbes, in this laboratory, noted the same defect. Apparently the thin vulcanite blade yielded sidewise somewhat during its rapid movement through the mercury. The defect was completely cured by Dodge by the expedient of inserting, within the mercury chamber in which the blade moves, a strip of watchspring, so adjusted as to press against the blade during its movement. I have substituted in my recent keys a glass vane $\frac{1}{32}$ inch thick, for the vulcanite. This has been tested by Forbes with the string galvanometer and shown to give clean breaks.

SUMMARY

1. Consideration of the conditions of distribution of stimulating currents through tissues leads to the suggestion that an inherent error of unknown amount, assumed to be 10 per cent, limits the degree of accuracy possible of attainment by quantitative stimulation methods, and, therefore, renders unnecessary for practical purposes extreme refinement of such methods.

2. The contention that approximating the coils of an inductorium modifies the contour of the shocks generated by it is

[19] Martin: This Journal, xxvi, 1910, 181.

shown to apply only to the inner portion of the field, within the direct influence of the iron core.

3. Physiological tests demonstrate that when the secondary coil is in the outer part of the field, beyond the direct influence of the iron core, the physiological efficiencies of break shocks generated by it are proportional to their contained currents.

4. Physiological tests demonstrate that a calibration scale for the outer part of the inductorium field, based upon contained currents, is valid when applied to tissues of high resistance as well as when applied to those of low resistance.

5. Physiological tests demonstrate that in the inner portion of the field a calibration that is valid for tissues of low resistance will not hold completely for tissues of high resistance. For practical purposes, however, the calibration is sufficiently accurate to be serviceable as far in as position 6 cm. in a "standard" inductorium.

6. Reference is made to a method, elsewhere described, for taking into account the influence of secondary resistance on the efficiency of induction shocks.

7. The calibration is shown by two distinct lines of evidence to apply to stimuli above the threshold as well as to stimuli at the threshold.

8. The *Nutzzeit* is considered as a possible factor modifying the validity of calibrations. Experimental evidence is offered that a calibration scale which is valid for muscle is equally valid for nerve, although the latter has a shorter *Nutzzeit* than the former. Comparisons of stimuli applied to tissues having different *Nutzzeiten* are admitted to be somewhat uncertain, but the belief is expressed that the error of such comparisons is probably no greater than the other errors inherent in the electrical stimulation of tissues.

9. In order for the shocks generated by different inductoria to be quantitatively comparable, the inductoria should be of reasonably similar construction. The Kronecker specifications are urged as suitable for adoption as standard.

10. A method is described of curing the defect that sometimes appears in the vulcanite knife-blade key. A glass blade is shown to be superior to one of vulcanite.

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The American Journal of Physiology is issued monthly by the American Physiological Society under the direction of the Council of the Society. There are at present from two to three volumes yearly, each of about five hundred pages. The price of one volume, postage free, is five dollars to subscribers in the United States and Canada and five dollars and twenty-five cents to subscribers in other countries. All subscriptions are payable in advance.

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CHANGES IN THE BLOOD AFTER MUSCULAR ACTIVITY AND DURING TRAINING

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Some years ago a number of investigators made a study of the changes wrought in the blood of man by muscular work. The majority of these determined only the changes in the number of the red or white corpuscles or in the specific gravity immediately at the close of the exercise and at one later period. In this study we have endeavored to follow the sequence of the changes in the blood's content of erythrocytes, total leucocytes, variety of leucocytes, platelets, and haemoglobin, and also the variations in the specific gravity, from the moment the exertion ended until equilibrium was again attained; and to find an explanation of these changes. In addition we have examined the blood of several athletes throughout a period of training to determine the effects of regular and frequently repeated muscular work.

The routine followed in each of our work experiments comprised a preliminary determination of the normal number of the several varieties of blood corpuscles, the percentage of haemoglobin, and the specific gravity of the blood. After this the subject either ran eight-tenths of a mile around a part of the college campus, or made a rapid run of 260 yards in a build-

ing in the course of which he ascended three flights of stairs, or worked for 15 or 20 minutes on a stationary bicycle. In several experiments the subject ran two miles on a cinder track. Immediately at the close of the exercise blood samples were taken from a finger so punctured that the blood flowed freely. At intervals of 15 minutes, or thereabout, other samples were drawn from fresh punctures until it was found that the normal condition of the blood was reëstablished. For these observations we had nine subjects and made thirty-six experiments.

The percentage of haemoglobin was determined with the Gower-Haldane haemoglobinometer and the counts of blood corpuscles were made with the Thoma-Zeiss haemocytometer. The red corpuscles were diluted with Hayem's solution, the white corpuscles with a 1 per cent solution of acetic acid, and the platelets with Wright and Kinnicutt's mixture of cresyl blue and potassium cyanide. In making the differential counts of the leucocytes Wright's triple stain was employed and two or three hundred of the corpuscles counted in at least two spreads of blood. For the specific gravity a series of wide-mouthed bottles containing mixtures of glycerine and water of different densities was employed.

PERCENTAGE OF CHANGES IN THE BLOOD

An increase in the number of red corpuscles occurs during muscular exertion. Willebrand (1) found this increase to vary between 2.9 and 23.4 per cent. Zuntz and Schumburg (2) in a study of German soldiers during marches obtained increases of 247,000 to 650,000 per cubic millimetre or an average increase of 9 per cent. Hawk (3) in his study of college athletes found the increase in the number of red corpuscles to vary from 400,000 to 1,460,000 or from 7.3 to 26.7 per cent. The minimum rise in the erythrocytes in our own experiments was 200,000 and the maximum 1,180,000. The percentage of increase varied between 3.2 and 22.8. The increase in the number of red corpuscles for a given exertion was not constant. One of our subjects ran eight-tenths of a mile twelve times,

the concentration in red corpuscles varied between 3.2 and 11.1 per cent. We believe these differences find an explanation in the distribution of the blood as determined by previous activity and stage of digestion.

The changes in haemoglobin, on the whole, were proportionate, but not always equal, to the increase in the number of the erythrocytes. The increase in our subjects varied between 3.5 and 10.9 per cent The maximum rise in haemoglogin was scarcely half the maximum for the red corpuscles. The parallelism was decidedly lacking in two experiments and in these the red corpuscles were far in excess. These two experiments gave our maximum figures for the rise in erythrocytes.

Hawk cites three investigations in which a leucocytosis was found after muscular work, the number of leucocytes varied from 11,400 to 22,200. His own observations on athletes showed the increase in the number of leucocytes to vary from 1,930 to 9,500 per cubic millimetre or from 21 to 104.4 per cent. Zuntz and Schumburg in their study of soldiers obtained an average leucocytosis of 43 per cent or an increase of 2,470 to 3,080 over the number found when the men were at rest. We, likewise, have found, following exercise, a corresponding increase in the number of white corpuscles; this increase varied from 1,170 to 11,670 while the percentage ranged from 13.8 to 130.2.

The influence of muscular activity upon the several kinds of leucocytes has been considered by Zuntz and Schumburg. They obtained an increase of polymorphonuclears of from 6 to 11 per cent and a decrease in the lymphocytes of from 3 to 17 per cent. Burrows (4), on the other hand, in a study of a single case found that exercise decreased the polymorphonuclear elements and increased the lymphocytes. Our own differential counts are invariably in accord with those of Zuntz and Schumburg and show an increase of from 9 to 45 per cent in polymorphonuclear elements and a decrease of from 14 to 55 per cent in the lymphocytes. Burrows found that convulsions increased the polymorphonuclears and decreased the lymphocytes and attempted to establish the fact that these changes were not due

to muscular activity but to a pathological condition. Our records correspond closely with the changes he found in these cases of convulsions and indicate that the blood changes observed by him after the convulsions may have been wholly due to the muscular activity of the attack.

We find no record of observations on the blood platelets following exercise. There was in our experiments a marked fall in the number per cubic millimetre, shortly after the cessation of muscular action, of from 17 to 30 per cent. This was later followed by an increase which carried the number above the normal 17 and more per cent.

The specific gravity of the blood in muscular activity has been carefully studied by Jones (5). He found that it usually varied directly with the red corpuscles. However, he reports that gentle exercise is accompanied by a fall, while the more prolonged or violent forms of exercise are accompanied by a rise in the specific gravity. Zuntz and Schumburg constantly obtained an increase in the specific gravity of the blood. The exercises used by us invariably caused a rise.

THE SEQUENCE IN THE CHANGES

We were unable to follow in every experiment all of the blood changes in which we were interested; nevertheless, in the large number of experiments made we have studied each change many times and in considerable detail. On several occasions the observations covered the six factors. The results of one of these experiments are given in the curves of figure 1. These show the sequence of the alterations in the blood as they were found to occur following a run of eight-tenths of a mile. The curves bring out rather clearly the fact that the changes in the number of erythrocytes, percentage of haemoglobin, and specific gravity are more or less parallel and suggest that they may be the result of a common causative factor. The curves showing changes in the number of leucocytes, lymphocytes, and platelets are placed together because of an association to be shown later.

The immediate effect of the exercises used was a concentration of the blood of the peripheral capillaries. Within a very few minutes after the completion of the exercise the blood began to

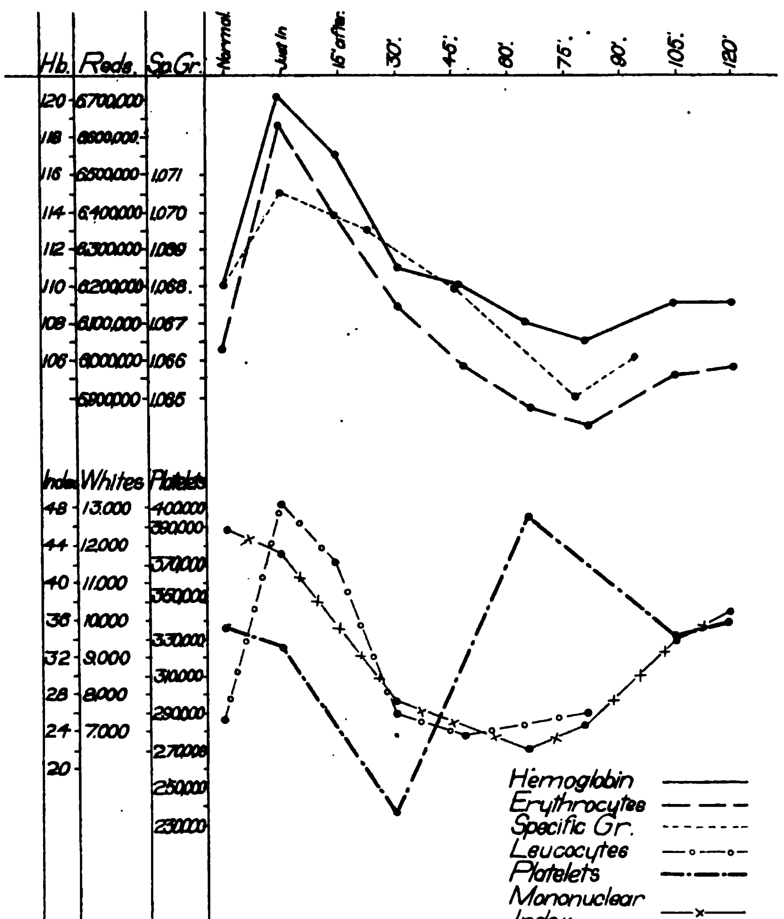

Fig. 1. The blood changes following a run of eight-tenths of a mile in 4.2 minutes.

dilute, and as a result the percentage of haemoglobin, the number of red corpuscles, and the density gradually diminished so that each returned to its normal figure in from 30 minutes to an hour and a quarter. The time required seemingly was

dependent upon the length of the period of muscular activity. However, the return to the normal condition of the blood was only temporary, the diluting process so continued that the composition with respect to haemoglobin, erythrocytes, and specific gravity soon became subnormal. The entire diluting process lasted from 50 minutes to 2 hours. After this the blood again concentrated and soon restored to the normal the three factors. The entire series of post-exercise changes was usually completed within 1 or 2 hours.

The maximum and minimum figures for the variations in haemoglobin, red corpuscles, and specific gravity in the various experiments showed some differences. In a number of instances the haemoglogin rose above and fell below the normal by an equal amount; in others the degree of dilution was less than the amount of concentration; while in four instances the subnormal stage did not appear. The percentage of change for haemoglobin and the number of red corpuscles was sometimes the same; for example, maximum haemoglobin 3.4, erythrocytes 3.2, minimum haemoglobin 3.4, and erythrocytes 3. Very frequently the percentage of change for the number of red corpuscles was greater than that for the haemoglobin as the following illustrates: maximum of haemoglobin 5.5 and of red corpuscles 6.9, minimum of former 5 and of latter 8, and another in which both the maximum and minimum for haemoglobin were 4.2 and for the red corpuscles 9.1.

The specific gravity varied directly with the red corpuscles, so that having determined the normal for both and the change in either the number of corpuscles or the specific gravity the amount of change for the other could be fairly closely predicted. The specific gravity variations were all similar to the following: (1) normal 1.068, maximum after exercise 1.0705, minimum 1.066; (2) normal 1.070, maximum 1.0715, minimum 1.069.

The form of the curve showing the sequence of the variations in the number of the leucocytes per cubic millimetre of blood does not correspond with those for haemoglobin, erythrocytes, and specific gravity. The percentage increase in leucocytes at the end of the exertion was usually far greater than it was

for these other factors and this gives a higher apex to the plotted curve. Usually the return to the normal number of white corpuscles was more abrupt, and occasionally slower, than was the case for haemoglobin or red corpuscles. Instances in which the changes were parallel were exceptional. The leucocyte count rarely showed a subnormal stage.

Differential counts of the leucocytes indicate a slow destruction of the lymphocytic elements of the blood following muscular work. Immediately at the close of the exertion there was, as a rule, no definite change in the proportions of the various kinds

TABLE 1

Differential counts after an eight-tenths mile run

	POLYNUCLEARS	SMALL LYMPHOCYTES	LARGE LYMPHOCYTES	LARGE MONONUCLEARS	TRANSITIONALS	EOSINOPHILES	MAST	INDEX
Normal	51.0	22.0	18.0	4.0	2.0	1.0	2.0	46.0
Just in	53.5	20.5	17.0	4.5	1.5	1.0	2.0	43.5
32 minutes	69.5	19.0	7.0	1.5	0	0	2.0	27.5
66 minutes	74.0	11.5	7.5	3.0	0.5	0	1.5	22.5
105 minutes	73.0	9.0	13.0	1.0	2.0	0	2.0	25.0
135 minutes	60.0	17.0	13.0	2.0	2.0	0	1.0	34.0
168 minutes	62.0	16.5	14.0	6.0	0.5	0.5	1.0	37.0
4 hours	50.0	32.0	10.0	5.0	1.0	1.0	1.0	48.0

of leucocytes. Slowly thereafter throughout a period of from 1 to 2 hours the polymorphonuclear elements increased and the lymphocyte index, which is obtained by adding the total number of mononuclears, the large and small lymphocytes, large mononuclears, and transitionals, decreased. The details of these changes as they were found in a typical experiment are given in Table I. The lymphocytic index was found to fall from 23 to 52 per cent. The following are examples of the total change in the index: 41.5 to 20, 39 to 26, and 42 to 26. The return to the normal proportions of the kinds of leucocytes was also gradual, requiring a period of from 2 to 4 hours. Dr. G. B. Gilbert informs us that the return to the normal lympho-

cyte index may be delayed as much as 24 hours after a night of dancing. The change in the proportion of the several varieties of leucocytes can not be accounted for by an influx of certain kinds from the tissues during exertion, since although the total number of leucocytes is enormously increased during the exercise the differential counts were, at the immediate close of work, either only very slightly or not at all altered. Further the changes in the differential counts continued long after the normal number of leucocytes per cubic millimetre had been restored.

The changes in the number of the blood platelets did not correspond to those of the other blood elements studied. Immediately at the close of muscular work, as was the case with the lymphocytes, it was found that there was either no change or a very slight decrease in the number. However, there then followed, apparently as a consequence of a destructive process, a rapid fall in the number which reduced the platelets within 30 to 60 minutes from approximately 350,000 to 250,000 per cubic millimetre. After this came a period of rapid increase which resulted in an over-production. Hence within an hour or two after exertion the number exceeded the normal from 17 to 25 per cent. Later as is shown in figure 1 there was a return to the normal number.

Hawk found in his study of various kinds of college athletic activities that the increase in the number of red corpuscles in the blood became gradually less pronounced as the exercise was continued. This he believed pointed toward the possibility of an actual decrease due to a rapid destruction of red corpuscles in prolonged violent exercise. We have five observations bearing on this point in which the subjects had walked a distance of 9.7 miles to the top of Pike's Peak, ascending approximately 7,900 feet. The average grade was 1 in 6 or 7, with some half dozen stretches of considerable length with a 25 per cent grade. The ascent was made in from 4 to 5 hours. It should be here noted that each man drank as much water as he desired during the climb. For three of the men only haemoglobin determinations were made, of these on arrival at the summit one showed

a drop from 112, his normal in Colorado Springs, to 108 or a fall of 3.6 per cent; the second gave no change, his normal 112 being obtained; the third had an increase from 111 to 116 or 4.5 per cent. Both the haemoglobin and the number of red corpuscles were determined for the other two subjects. One gave a haemoglobin reading of 116 and had 6,514,000 red corpuscles per cubic millimetre in Colorado Springs, while upon arrival at the summit the haemoglobin was 127, or concentrated 9.5 per cent, and the red corpuscles 6,920,000, an increase of 16.2 per cent. The fifth man had in Colorado Springs a haemoglobin content of 112 and 6,224,000 red corpuscles, arriving on Pike's Peak these had increased to 128 or 14.3 per cent and to 6,872,000 or 10.4 per cent respectively. The after-diluting process which soon produces a subnormal condition, occurred in each subject but could not be followed to the end because of lack of proper light for titrating the blood. In the last two subjects observations throughout a period of 4 hours failed to show the process of dilution completed.

These results, taken as a whole, do not support Hawk's theory that there is an increased destruction of erythrocytes during muscular activity. The work accomplished was severe and long continued and thus gave ample time for destruction of corpuscles. Nevertheless, in only one of the five men was there a suggestion of such destruction. Our subject who showed the lowered content of haemoglobin was known to be the least fit physically. Circulatory observations, such as arterial, capillary, and venous pressures, and rate of blood flow, proved that he did not react as well as physically strong men. Evidence, which is given later, suggests that the decrease in the haemoglobin and red corpuscles was not due to an actual destruction of corpuscles but rather to their stagnation in some of the capillaries because of fatigue.

Zuntz and Schumburg found the blood concentrated in soldiers who had marched for seven hours over a course of 24.75 kilometres carrying loads of from 27 to 31 kilos.

THE EXPLANATION OF THE BLOOD CHANGES

Hawk advances six possible explanations for the increase of erythrocytes in exercise. These are (1) the production of new corpuscles, (2) concentration of the blood through increased urine formation and copious sweating, (3) concentration of the blood through increased evaporation in the lungs, (4) passage of fluid from the blood to the active muscles, (5) concentration of the blood through vaso-motor contraction and rise in blood pressure, and (6) sudden passage into the blood of a large number of cells lying dormant in various parts of the body. He is inclined to accept the last factor as the primary cause of the increase in red corpuscles since in a short run, such as a hundred yard dash, involving only a few seconds in time there would not be sufficient opportunity for any of the first five factors to accomplish the degree of concentration observed. As exercise is continued the factors of copious sweating, accelerated urine formation, increased evaporation from the lungs, and the passage of fluid from the blood to the working muscle, all help to keep up the concentrated condition of the blood and so obscure his predicated opposite action, namely an accelerated destruction of red corpuscles. Willebrand believes that the withdrawal of water from the blood by the working muscles is the primary cause of the concentration. Zuntz and Schumburg accept Willebrand's explanation.

To account for the leucocytosis found to be present after muscular activity, Hawk adds another to the six factors mentioned above; it is that there is a changed distribution of the leucocytes followed by their accumulation in the peripheral circulation. This he believes is the primary cause of leucocytosis, which he further explains by the fact that greatly increased rapidity of circulation would throw out from the interior into the general circulation a great number of leucocytes which arriving in the peripheral arterioles and venules would lag behind the general blood stream and produce an apparent leucocytosis of large dimensions. Zuntz and Schumburg believe since the white corpuscles increase so much more than the red that

a different explanation must obtain for their increase. They hold that the passing of wandering cells from the tissues into the general circulation is an adequate explanation.

We have attempted in several series of experiments to determine the importance of the various explanations offered for the changes in the blood during exertion. That there is a stagnation of red corpuscles which withdraws large numbers from the general circulation is indicated in the following observation. In this experiment the subject ran eight-tenths of a mile in 4 minutes and 50 seconds. His blood was then examined at intervals until found to be well down in the subnormal stage when he once more ran, in 5½ minutes, the same distance. The results follow:

	HAEMOGLOBIN	RED CORPUSCLES
	per cent	*millions*
Normal	117	6.4
1 minute after first run	125	7.2
23 minutes after first run	119	
35 minutes after first run	115	6.2
57 minutes after first run	114	
60 minutes ran second time		
1 minute after second run	123	7.1
17 minutes after second run	118	6.5
30 minutes after second run	116	
47 minutes after second run	111	5.9
83 minutes after second run	111	5.9
120 minutes after second run	112	

As a result of the first run the haemoglobin content had increased 6.8 and the number of red corpuscles 12.5 per cent; in the second the haemoglobin again rose above the normal 5.1 and the red corpuscles 10.9 per cent, while they were 7.9 and 14.5 per cent respectively above their content at the start of the second run. The return of both, during the second run, to almost the values obtained after the first makes it probable that we are not here dealing with a loss of water from the body but only with a side-tracking of a large mass of corpuscles.

Abdominal pressure experiments. Mitchell (6) reported that in a healthy subject general massage increased the number of

red corpuscles and to a lesser degree, or sometimes not at all, the haemoglobin. He obtained in a case of chronic lead poisoning an increase in the erythrocytes from 4,000,000 to 4,500,000 with the application of abdominal massage for 25 minutes. This observation points to the splanchnic area as a large source of reserve corpuscles and led us to test the influence of abdominal massage. It early became apparent that prolonged abdominal massage was not necessary to bring about the rise, so instead a heavy weight of lead foil, weighing 25 pounds and so shaped as to fit between the lower ribs and the top of the pelvis, was placed on the abdomen of the reclining

TABLE 2

Weight-massage of the abdomen

SUBJECT	TIME	HAEMOGLOBIN	ERYTHROCYTES	LEUCOCYTES
L.C.H.	Normal	115	6,328,000	9,600
	5 minutes	120	6,876,000	10,200
	20 minutes	119	6,856,000	10,000
E.C.S.	Normal	106	5,240,000	8,000
	4 minutes	108	5,432,000	8,400
	16 minutes	110	5,640,000	8,300
D.L.S.	Normal	116	6,392,000	
	4 minutes	119	6,688,000	
	19 minutes	123	6,976,000	

subject. The act of breathing moved the weight slightly and gave a massage effect with the result that in 2 or 3 minutes an increase in haemoglobin and red corpuscles was evident in the peripheral circulation. Such experiments were prolonged 10 to 30 minutes. Frequently the maximum change in the blood occurred within 5 minutes but in others was delayed as much as 20 minutes. We used six subjects and each several times for these observations. The data for three typical experiments are given in table 2. The average increase for all of our observations was haemoglobin 5.1 and red corpuscles 8.7 per cent. These figures are strikingly close to the average of the increases obtained from all of our work experiments in which the average increase in haemoglobin was 6.3 and in red corpuscles 9.6 per

cent. Immediately after a meal and while digestion was at its height abdominal pressure failed to alter the blood.

Prevention of return to normal. If the splanchnic area is the chief reservoir of reserve red corpuscles it may be that pressure on the abdomen after exertion, by delaying side-tracking, will prevent the return to the normal content of haemoglobin and erythrocytes. To test this two methods were employed, the first, which was the least effective, was to draw a belt around the abdomen as tightly as possible within three minutes after the return from a run; the second was to have the subject take the reclining posture with the lead weight on the abdomen. Both methods were always effective in preventing to a considerable degree the dilution. Thus in one experiment by use of the tight belt with the subject standing, after a run of eight-tenths of a mile had increased the haemoglobin from 113 to 118 and the red corpuscles from 5,840,000 to 6,560,000, at the end of an hour and a half the haemoglobin was still 115 and the erythrocyte count 6,144,000. A protocol of an experiment for which the subject ran a half mile in 3 minutes and 58 seconds and afterward reclined with a weight resting on his abdomen follows:

	HAEMOGLOBIN	RED CORPUSCLES
Normal	107	5,680,000
Just in	115	6,360,000
19 minutes later	114	
39 minutes later	113	6,328,000
61 minutes later	111	6,240,000
85 minutes later	111	6,100,000
23 minutes after the weight was removed	107	5,696,000

Curves have been plotted for this experiment in figure 2 and placed in contrast with curves from a typical experiment when the diluting process was not interfered with. The curve for the typical reaction returns to the normal level and passes into the subnormal phase in about 50 minutes, while that expressing the condition in which abdominal pressure was employed only descends half way to the normal in 85 minutes. It is improbable that either method used for preventing the stagnation of

blood in the splanchic area is completely effective hence we conclude that the splanchnic area is the primary source of the reserve of red corpuscles thrown into the general circulation during muscular activity.

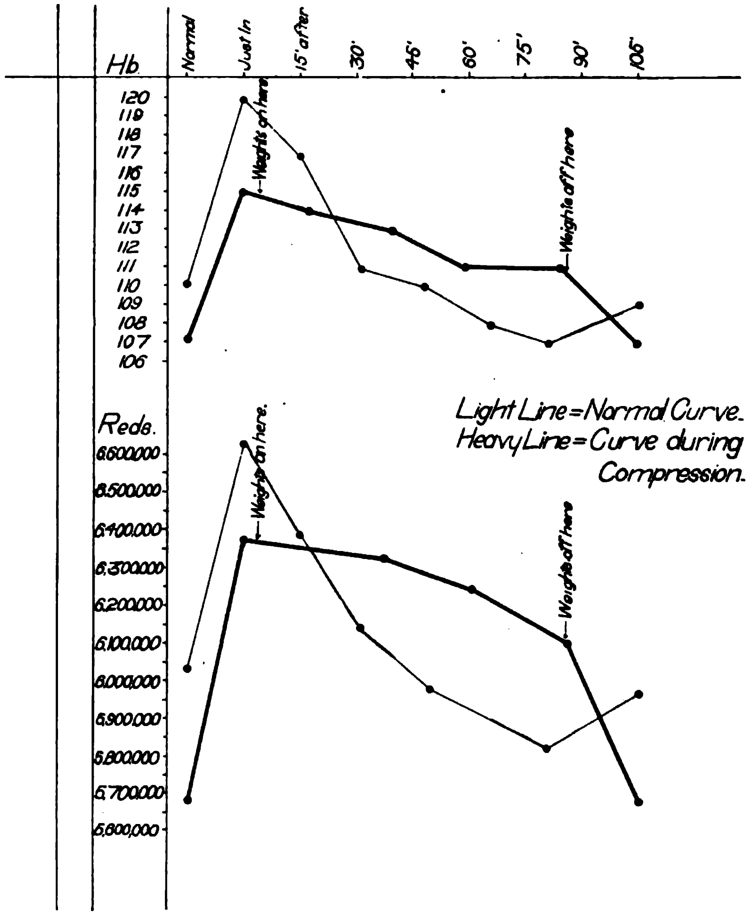

Fig. 2. A comparison of the changes in the haemoglobin and red corpuscles after runs of eight-tenths of a mile following which the abdomen was in one instance compressed and in the other not under pressure.

Work and abdominal pressure experiments at a high altitude. Two series of observations made upon the summit of Pike's Peak, on subjects who had sojourned there from 3 to 10

days, strengthen the above conclusions. In one series six subjects did muscular work either by making a run of 175 yards up a path of considerable grade, a part being a 25 per cent incline, or ran about a half mile on the level, or worked for about 15 minutes on a stationary bicycle. In eleven experiments the customary rise in haemoglobin, red corpuscles, and specific gravity did not occur. While in a majority immediately at the close of exertion the blood had not changed, in three instances it had diluted 2 and more per cent. In each work experiment the post-diluting process appeared as at the lower altitude. A typical protocol for a half mile run is given below:

	HAEMOGLOBIN	ERYTHROCYTES	SPECIFIC GRAVITY	PLATELETS	LEUCOCYTES
		millions			
Normal	126	6.9	1.0665	360,000	6,500
Just in	126	6.9	1.0660		10,200
30 minutes later	124	6.6	1.0645	280,000	
55 minutes later	118	6.2	1.6400		
80 minutes later	120	6.4			

This lack of increase of haemoglobin and erythrocytes during physical exertion at the high altitude invalidates Willebrand's explanation of the concentration, which is also accepted by Zuntz and Schumburg. They believed that the blood was concentrated in exercise by the passage of fluid from the blood to the active muscles. If this explanation is correct the blood, during exertion, should have been concentrated as much on Pike's Peak as in Colorado Springs. These observations also show that loss of water from the body by evaporation from the lungs, and by increased sweating and urine formation is not an adequate explanation of the concentration that occurs at the low altitudes.

A series of ten abdominal pressure experiments was made in the same manner as at the lower altitude and in several the abdomen was also vigorously kneaded for some minutes before the weights were placed in position. The striking feature of these experiments was an opposite reaction from that obtained by a similar procedure at the low altitude, instead of an increase

in haemoglobin and red corpuscles a reduction in both of from 2 to 4 per cent was obtained. The following is a typical result from an experiment in which both kneading and pressure were employed:

	HAEMOGLOBIN	RED CORPUSCLES
Normal	123	6,744,000
Pressure 5 minutes	121	6,568,000
Pressure 13 minutes	120	6,496,000

These massage-pressure experiments show clearly why the blood failed to concentrate during exercise on Pike's Peak. The reserve of corpuscles had been depleted because of the great need of oxygen at the high altitude. At low altitudes the need for a greater supply of oxygen occurs only during muscular activity; at high altitudes the need is also felt during rest, with the result that even at the beginning of muscular work there are no corpuscles remaining in reserve, and therefore there is no increase with exercise.

The influence of adrenalin. Having found evidence that the splanchnic area is an important and very likely primary reservoir for the superabundant red corpuscles during inaction, the question arose as to how the extra supply was thrown into the general circulation during exercise. In view of the recent researches by Canon (7) and his pupils which show the relation of the adrenal medulla to muscular activity; and in view of the fact that v. Anrep (8) had shown that an increase of carbon dioxide in the blood, such as occurs in exercise, by stimulating certain nerve centers, caused the suprarenals to secrete more freely; and because it was well known that the circulation of the abdomen is shifted to the general circulation by this secretion, it occurred to us that we were dealing with another of the adaptive functions regulated by adrenalin. We, therefore, administered by mouth to a subject, when the stomach was empty, a Parke, Davis and Company preparation of adrenalin with the following results:

	HAEMOGLOBIN	ERYTHROCYTES	LEUCOCYTES
45 drops of adrenalin administered at 11.34 a.m.			
Normal	115	6,064,000	8,600
In 10 minutes	116	6,272,000	6,800
In 20 minutes	119	6,440,000	7,800
In 30 minutes	118	6,280,000	8,400
16 drops more administered 34 minutes later.			
40 minutes after first dose	121	6,472,000	
55 minutes after first dose	118	6,296,000	
70 minutes after first dose	117	6,176,000	

The first effect of adrenalin so administered would no doubt be a vaso-constriction of the splanchnic area and, as Edwards (9) has recently shown in a study of the compensatory distribution of the blood during stimulation of the splanchnic nerve, there would be an increased transfer of blood to the vascular circuits of the head and extremities sufficient to compensate for the diminished flow through the portal circuit. If then a large mass of red corpuscles has been side-tracked in the splanchnic area and blood plasma withdrawn, the constriction of the vessels of this large vascular area would force the stagnant mass of red corpuscles on toward the heart into the general circulation and thus give the increase in haemoglobin and red corpuscles found in the above record.

How account for the changes in the number of leucocytes and platelets? Several fragmentary observations show clearly that the changes occurring in the leucocytes and platelets as a result of physical exertion are not produced in the same manner as those in the haemoglobin, red corpuscles, and specific gravity. In three experiments on Pike's Peak the changes in the number of leucocytes and platelets were followed through a part of the process and they were found to react exactly as at the lower altitude. The white corpuscles increased as much as 57 per cent in a run, and the platelets decreased fully 28 per cent, while they later showed the customary overproduction. Thus we here find proof for the contention of other workers that the increase

in leucocytes in exercise can not be the result of the same reaction that increases the number of red corpuscles. At high altitudes exercise squeezes the lymph out of the muscles and drives it toward the heart just as at low altitudes, and with it would be carried the leucocytes gathered from the tissues. The supposition that the leucocytes are derived from the tissues other than the splanchnic area was further supported by the abdominal pressure determinations made in Colorado Springs, some of which are recorded in table 2. In these the leucocytes were only increased from 4 to 7 per cent, while in exercise they increased 14 to 130 per cent.

Some connection appears to exist between the mononuclear or lymphocyte index and the variation in the number of platelets. For some minutes after the exertion there appears to be a destruction of platelets which in turn very likely leads to a disappearance of the lymphocyte elements. Brown (10) has shown when an excessive demand for platelets exists that the mononuclear and transitional cells in the marrow, spleen, and blood may form platelets. Here then is a possible explanation for the decrease in lymphocytes.

The picture of the blood changes in exercise may be briefly summarized. During muscular inaction a large mass of the blood is diverted to the splanchnic area, where it probably stagnates and gives up plasma as lymph. There is also throughout the remainder of the body, especially in the limbs, an accumulation of lymph. With the onset of muscular activity the carbon dioxide content of the blood rises, this carbon dioxide stimulates the central nervous centers which regulate the secretion of the suprarenal glands, hence the output of adrenalin is increased. The adrenalin causes a constriction of the blood vessels of the splanchnic area, this forces the stagnant red corpuscles into the general circulation, thus giving the rise in specific gravity, haemoglobin, erythrocyte, and leucocyte content of the peripheral blood. Further the contraction of the voluntary muscles accelerates the flow of lymph, throwing lymph rich in leucocytes into the blood. The increase in red corpuscles and haemoglobin makes it possible to supply more readily the greater

demand for oxygen made by the active muscles. Shortly after the close of the exercise the carbon dioxide content of the blood falls below normal, as a result the discharge of adrenalin becomes subnormal and the blood once more accumulates in the splanchnic area, so that there is a gradual return to the normal composition and even a temporary subnormal content in red corpuscles.

BLOOD CHANGES DURING A PERIOD OF ATHLETIC TRAINING

Some data has been accumulated to show the influence of training upon the blood. This phase of the work was disappointing since we were unable to secure the hearty coöperation of the subjects that was necessary to furnish a complete picture of changes. We had only three men who were available both before they began training and during the season of spring athletics. One subject who trained for the two mile run was followed closely, the others gave opportunity for only a few observations during each period. Briefly, the results of these studies show an increase in haemoglobin and red corpuscles during the period of training for two of the subjects and no change in the third, For the subject followed closely the average content of haemoglobin in the foreperiod was 111.9 and during the height of training 115.8, which is an increase of 3.6 per cent. The red corpuscles averaged in the foreperiod 6.2 millions and 6.5 millions per cubic millimetre while in training, or an increase of 4.8 per cent.. The second subject showed an average increase of 9.4 per cent in haemoglobin, this rose, as a result of training, from an average of 117 to 128. The erythrocytes increased from 6.1 to 7.2 millions or 18 per cent. No change was found to occur in the number of leucocytes and platelets. For the subject followed most carefully differential counts of the leucocytes were made in sufficient numbers in both periods to permit a comparison. There was an increase in the lymphocyte index of 4.1 per cent. In the untrained condition the lymphocyte index averaged 37, while during the period of splendid condition it was 38.75.

A series of determinations of the total oxygen capacity of the blood and total blood volume was made on the one subject closely studied. The results of this study appear in Table 3. The determinations were made by the carbon monoxide method of Haldane and Lorrain Smith (11).

No change in total oxygen capacity and blood volume is shown by these determinations. With the haemoglobin of this subject's blood only increased 3.6 per cent, it is unlikely that the method of determining total oxygen capacity is exact enough to detect so small a change.

TABLE 3 (SUBJECT I)

DATE	PERCENTAGE OF HAEMOGLOBIN	PERCENTAGE OXYGEN CAPACITY	TOTAL OXYGEN CAPACITY	TOTAL VOLUME OF BLOOD
			cc.	cc.
February 13	113	20.9	1032	4938
February 20	109	20.2	1039	5144
February 27	109	20.2	1003	4965
March 24[1]	113	20.9	1037	4962
April 17	112	20.7	1045	5048
May 3	116	21.5	1057	4916

[1] Training began March 1.

SUMMARY

1. The immediate influence of physical exertion upon the blood of the peripheral capillaries was one of concentration in which the percentage of increase varied as follows: haemoglobin 3.5 to 10.9, erythrocytes 3.2 to 22.8, and leucocytes 13.8 to 130.2. The specific gravity increased proportionately with the red corpuscles.

2. Within a few minutes after the close of the exertion the blood began to be diluted and this usually resulted in a subnormal specific gravity and content of haemoglobin and red corpuscles.

3. At the end of the exertion the differential counts of the leucocytes showed no change, but very soon the polymorphonuclears increased 9 to 45 per cent and the total number of mononuclear elements decreased 14 to 55 per cent.

4. The number of platelets was unchanged at the close of the exercise. However, there then followed a period of decrease which reduced them 17 to 30 per cent. Later there was a period of overproduction which carried the number above the normal 17 to 25 per cent.

5. Long continued and severe exertion apparently does not cause an increased destruction of red corpuscles.

6. A repetition of a run when the blood has passed into the subnormal condition practically restores the maximum concentration.

7. Abdominal massage and pressure raise the content of haemoglobin and red corpuscles in the blood of the peripheral capillaries.

8. A tightly drawn belt or pressure on the abdomen following exertion prevents in large measure the dilution of the blood.

9. At an altitude of 14,109 feet the blood does not concentrate during exercise. However, the changes in white corpuscles and platelets occur as at the lower altitude.

10. Abdominal massage and compression cause a dilution of the blood at the high altitude.

11. Adrenalin administered by mouth causes the blood in the peripheral capillaries to be concentrated.

12. During a period of training for spring athletics the percentage of haemoglobin and the number of red corpuscles were found to increase. The number of leucocytes and platelets was not changed. The mononuclear elements were increased 4.1 per cent. No change in total oxygen capacity or total blood volume was found.

BIBLIOGRAPHY

(1) Willebrand 1903 Skandinavisches Archiv für Physiologie, xiv, p. 176.
(2) Zuntz and Schumburg 1901 Physiologie des Marches.
(3) Hawk 1904 This Journal, x, p. 384.
(4) Burrows 1899 American Journal of the Medical Sciences, cxvii, p. 516.
(5) Jones 1891 Journal of Physiology, xii, p. 309.
(6) Mitchell 1894 American Journal of the Medical Sciences, cvii, p. 502.
(7) Canon 1914 This Journal, xxxiii, p. 356.
(8) V. Anrep 1913 Journal of Physiology, xlv, p. 318.
(9) Edwards 1914 This journal, xxxv, p. 15.
(10) Brown 1913 Journal of Experimental Medicine, xviii, p. 278.
(11) Haldane and Lorrain Smith 1900 Journal of Physiology, xxv, p. 331.

THE NEUTRALIZING POWER OF SALIVA IN ITS RELATION TO DENTAL CARIES

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A cursory examination of the dental literature of the last few years shows one topic—the influence of the salivary secretion upon dental caries—to be of vital interest, a topic often discussed and about which many articles have been written.

The importance of the saliva in the etiology of dental caries is a well established fact, but the variability in the composition of the secretion has rendered the exact study of the saliva difficult.

The history of the work done and the theories advanced, controverted, and again advanced, is voluminous. The whole subject, in fact, has been so well discussed by others that a repetition here or references to all authors would involve a resumé which seems at this time unwarranted. The omission of many names then, is not prompted by lack of courtesy, but from the desire to omit all possible extraneous material and only those authors are mentioned whose work has a direct bearing on the problem in hand.

The different phases of the subject which have been under discussion at various times are numerous. In Johnson's (2) Operative Dentistry is found an elaborate theory by G. W. Cook in which he undertakes to prove that mucic acid may be found in the mouth and that it is responsible for many eroded areas on the tooth structure. His suggestion, however, is insufficiently supported by scientific evidence. According to our present day knowledge of physiology and pathology, mucic acid never occurs in the human body. Rose (14) has shown that mucic

acid is not an intermediary product in the metabolism of galactose-yielding sugars. Furthermore Thierfelder (15) working on the isomer of mucic acid, saccharic acid, says that although this acid results from the oxidation of glycuronic acid in vitro there is no evidence that this change can take place in the animal organism.

Pickerill (3) has attacked the problem from the standpoint of reaction to different stimuli and has shown in tabular form the increase in alkalinity of a saliva which has been activated by acid substances.

Important contributions have been made by Gies (4) and his co-workers. In a series of carefully conducted experiments covering over three years they have given us results which have thrown much needed light on the problem in hand.

He fails to find a definite relation between the general composition of the saliva and the condition of the teeth and notes that the systemic condition of the patient has a direct bearing upon susceptibility to dental caries. Bunting (5), Bödecker (6), Howe (8) and others have given us the benefit of their theories but unfortunately they fail in nearly every instance to describe their experiments and their theories can hardly be regarded seriously without such detail.

Kirk (7) brings out some important data in relation to indicators in the titration of saliva, by comparing results obtained with methyl orange, congo-red, litmus, phenolphthalein and thymolphthalein. He defines Pickerill's *index of alkalinity* as an *index of the power of the mucin to clear the saliva of its acid content* by forming an acid mucin coagulum. In fact he seriously questions the reliability of Pickerill's results pointing out quite definitely that the use of methyl orange in weak solution depends too much upon the personal equation to insure the obtaining of trustworthy data.

The reaction of saliva is amphoteric, that is to say it exhibits the power of neutralizing both acids and bases. Many of the body fluids show this phenomenon. Fresh milk is amphoteric; urine is often amphoteric to litmus, i.e., it will show both an acid and alkaline reaction to that indicator. Sutton (11) says "Saliva

which is generally neutral to litmus is always strongly alkaline to lacmoid or congo-red and acid to tumeric." The indicator-method of neutrality determination does not show the exact neutrality point. The method is comparable with itself and not with the electrolytic method of determining neutrality.

The alkalinity of the saliva has been variously stated as being due to dibasic sodium phosphate and also sodium carbonate. Raymond (12) quotes Chittenden and Richards (13) as saying that "*Human saliva contains normally no sodium carbonate whatever* the alkalinity, as indicated by litmus, lacmoid, et cetera, is due to hydrogen alkali phosphates (and is equivalent to 0.08 to 0.10 per cent Na_2CO_3) and to possibly some alkali bicarbonate. Mixed saliva invariably reacts acid to phenolphthalein." Taylor (16), on the other hand, claims that "Fresh saliva is quite neutral but becomes faintly alkaline as the CO_2 is dissipated, later it becomes acid again through bacterial action." Allaria (18) states that his experiments "Prove the saliva from breast-fed babies to be acid." Gies (4) reports at the inception of his work that "In practically every instance the reaction was alkaline to both litmus and lacmoid and in every case was acid to phenolphthalein." All of Gies' reports express acidity.

There seems to be a little confusion among the different experimenters in regard to the results obtained. This may be due to a lack of observance of standard conditions in obtaining the samples or to different indicators used.

The choice of indicators in this work is not large. The dissolved gases, as CO_2, precludes those indicators which are affected by that substance. Boiling the sample to rid it of this gas is unwarranted for the proteids are thereby coagulated. The indicators chosen should possess the greatest sensibility, should show a definite color change, and should change at the point nearest the hydrogen ion concentration of water, viz. H^{10-7}. Phenolphthalein possesses these requirements changing between H^{10-8} and H^{10-9}; to it saliva reacts acid with the exception of a few instances to be mentioned later. Para-nitro-phenol changing between H^{10-6} and H^{10-7} was chosen as the second indicator and, although the color change is not so satisfactory as that of phenol-

phthalein, still, when the ammonium salt is formed in titration, the yellow color shows very definitely against the turbid saliva. Methyl red was used at first but was discarded later in preference to para-nitro-phenol. Methyl orange was not used on account of the indefinite color change in solutions weaker than $\frac{N}{10}$. Both Sutton (11) and Treadwell-Hall (22) question the accuracy of methyl orange in weak solutions.

The following solutions were prepared: HCl $\frac{N}{10}$, NaOH $\frac{N}{10}$, NH_4OH $\frac{N}{10}$. These were carefully standardized and the standardization confirmed every two weeks. From these tenth normal solutions two hundredth normal were made by diluting 50 cc. of $\frac{N}{10}$ to 1 litre. This strength of solution was used throughout the work. These also were standardized every two weeks against the $\frac{N}{10}$ solutions.

All reports are based on 10 cc. of saliva as samples and titration figures indicate the number of cubic centimeters of $\frac{N}{200}$ HCl and $\frac{N}{200}$ NaOH. Temperature conversion factors were computed for every 4°C. but as the temperature variation was within 10°C. during the entire experiment it was found unnecessary to correct for this variation in the use of $\frac{N}{200}$ solutions.

The normal resting saliva is a relative term. We have no means of knowing what is normal or what is resting. Conditions in the mouth vary not only from day to day but probably from hour to hour. Whether this is due to local conditions in the mouth entirely is an open question. The psychic influences are constantly changing and how much this affects the secretion of the saliva we may judge from the classical experiments performed by Pavlov (9) and others. Pavlov has shown that even the rattling of a cart in the street below will excite the salivary secretion of a dog. Heidenhain also has shown that by the use of weak induction shocks to the *chorda tympani* nerve he could cause the submaxillary glands to secrete a thin copious saliva. By stimulation of the *sympathetic* the flow was changed and became thick and turbid. These experiments explain to a certain extent the difficulties encountered in obtaining a "normal resting saliva," and the consequent variation in analyses of saliva from the same individual. The determination of a constant

normal resting saliva involves, at present too many x's in the equation and has not been made, so far as the writer is aware.

The samples of saliva for this research work were obtained largely through the kindness of the authorities of various hospitals, asylums and penal institutions. Certain inmates gladly coöperated, after explanations, in furthering the work of the writer. In all 104 cases were examined, but voluntary coöperation was obtained in only 96 cases.

Following the suggestion of Dr. Gies (4) the same type of bottle was used in the collection of every sample—a 2 oz. glass stoppered "salt mouthed" bottle. The subject, (or more correctly, the person), was requested to expectorate in the bottle without making a conscious effort to produce the saliva. The bottle was kept tightly closed during the intervals which elapsed between the actual acts of expectorating. Before the sample was collected the condition of the mouth was noted, also any habits in regard to tobacco, alcohol or diet. Also the following points were observed:

1. The samples to be comparative throughout were obtained by natural methods or by the ordinary methods of stimulation; or, if in the resting stage, under known, or at least as constant conditions, as possible.

2. The patient should be as nearly normal as possible in salivary activity.

The data obtained from the several hospitals were compiled only from those convalescent patients who were not taking any medication (except as noted). The data from the prison inmates were compiled from individuals who were in good health and who were doing the regular prison routine work. The data from the inmates of the insane asylums were taken from those who were not entirely mentally deficient for the obvious reason that it was only that class which could be made to understand what was wanted. The diets which were carefully studied and compared throw little light upon the results from the standpoint of Pickerill's work, for, in one instance, where the carbohydrate content is in excess one would almost expect, judging from the results of Pickerill (3), to find a higher acidity than is reported.

METHODS OF ANALYSIS

For Alkalinity. Ten grams of the well mixed sample are placed in a casserole diluted with 20 cc. of distilled water at 20°C. and free from CO_2. About eight drops of an aqueous solution of para-nitro-phenol are added and $\frac{N}{200}$ HCl run in, in slight excess. The mucin is thereby precipitated and the determination of the end point made easier in the clarified solution. Next $\frac{N}{200}$ NH_4OH is added until the first faint trace of a yellow color is discernible. The difference between the amount of acid added in the first instance and the amount of alkali added in the second, equals the alkalinity in terms of cubic centimeters of $\frac{N}{200}$ HCl and is thus reported.

For Acidity. A second 10-gram sample is diluted as above and about four drops of phenolphtbalein are added. Then $\frac{N}{200}$ NaOH is run in until the first permanent pink color appears. The cubic centimeters of alkali added equals the acidity of the sample in terms of cubic centimeters of $\frac{N}{200}$ NaOH and are thus reported.

The double titration in the determination of alkalinity while not absolutely necessary, is advisable as it makes for accuracy. For instance the HCl may be added to a point of decoloration of the indicator but this exact point is obscured in many instances by the color of the sample itself. With the precipitation of the mucin, by the addition of the excess of acid, this difficulty is overcome.

Since phenolphthalein does not react satisfactorily with ammonia it was necessary to employ NaOH in the determination of acidity, while the NaOH used in conjunction with the para-nitro-phenol was not so satisfactory as NH_4OH for the reason that the latter gives a more definite end point with this indicator.

In the analyses of samples from those institutions outside of San Francisco the apparatus and solutions were taken and analyses made at the respective places at the time of taking the sample. This insured a direct comparison of all analyses presented, since only one of the samples was allowed to stand for longer than an hour before titrating.

The following were the results obtained (tables 1–8). In the tables the word "immunity" is used in the sense of freedom from caries. Those cases which on careful examination showed no fillings and no areas of decay have been referred to as "absolute immunity." Those cases which showed fillings but not areas of decay are referred to as "present immunity." The term "with care," denotes that there is evidence of the patient using the toothbrush, i.e., "caries with care," denotes that a carious condition exists at the time of taking the sample, and that the patient cleanses the mouth and endeavors to keep the teeth as free from food débris as possible.

TABLE I

Present immunity, with care

NUMBER OF PATIENT	cc. $\frac{N}{200}$ HCl	cc. $\frac{N}{200}$ NaOH	NUMBER OF PATIENT	cc. $\frac{N}{200}$ HCl	cc. $\frac{N}{200}$ NaOH
1	23.00	4.50	3	9.94	4.82
	25.50	4.30		8.94	5.02
	28.40	4.90		9.20	5.30
	16.02	5.86		18.10	3.40
	16.80	6.34			
	12.06	5.72	11	10.70	5.80
	13.70	5.42		11.70	5.45
	20.20	6.42			
			14	7.58	18.30
2	11.00	12.60			
	13.70	14.60	36	21.64	4.41
	14.06	13.10	40	9.90	8.00
	11.02	10.50	41	17.04	11.22
	7.36	12.34	55	9.04	4.02
	9.40	9.46	61	9.50	4.30
	10.72	9.34	65	10.60	9.86
	8.32	12.10	67	13.70	4.97
	12.90	8.20	70	17.90	7.78
				14.45	5.10
3	17.70	4.50	74	19.80	8.00
	19.50	5.60	75	26.60	4.30
	17.50	5.40	89	24.25	2.70
	12.50	5.00	103	7.80	7.53

In table 1 is presented the results of the titration of "normal resting saliva" collected from the mouths of those patients who habitually take good care of their teeth and who at present show

no signs of caries. In the case of the first three patients the tests extended over a period of days. The samples were taken at the same time each day and titrated at once. The wide variation shown not only between the several patients but also daily by the same patient argues against the theory that acidity of the *resting saliva,* (that is of the saliva which is contaminated with the microorganisms of the mouth and food débris,) is an influencing factor in the *causation* or *progress* of dental caries, and also against the view that the alkalinity of resting saliva acts as a protecting

TABLE II

Present immunity, without care

NUMBER OF PATIENT	cc. $\frac{N}{200}$ HCl	cc. $\frac{N}{200}$ NaOH	NUMBER OF PATIENT	cc. $\frac{N}{200}$ HCl	cc. $\frac{N}{200}$ NaOH
5	24.60	5.60	50	7.14	7.52
	24.70	7.20	51	11.32	5.54
	22.20	9.80	53	1.82	12.02
	19.30	14.40	54	12.52	5.80
			58	21.10	alkaline (trace)
8	10.30	9.26	59	11.86	6.00
	14.08	11.86	64	11.40	8.67
	14.20	4.50	72	29.10	3.44
	22.22	7.60		22.50	6.28
				29.10	3.44
32	8.96	7.20	73	9.75	15.60
43	15.14	4.80	79	16.50	2.13
46	13.38	5.10	83	21.55	7.00
48	17.26	2.98	96	28.80	6.97
49	2.66	7.62	97	25.60	4.45

agent against caries. It will be shown later, however, that the *activated saliva* produced under definite stimulation, *is* an influencing factor in the production and maintenance of immunity.

For instance:

In patient No. 2 Table 1 the variation is 6.70 cc. HCl; 6.40 cc. NaOH.
In patient No. 3 Table 1 the variation is 9.56 cc. HCl; 1.20 cc. NaOH.
In patient No. 5 Table 2 the variation is 5.40 cc. HCl; 9.80 cc. NaOH.
In patient No. 8 Table 2 the variation is 11.92 cc. HCl; 7.06 cc. NaOH.
In patient No. 4 Table 3 the variation is 8.58 cc. HCl; 3.57 cc. NaOH.
In patient No. 6 Table 4 the variation is 5.60 cc. HCl; 1.30 cc. NaOH.
In patient No. 20 Table 4 the variation is 16.20 cc. HCl.; 8.02 cc. NaOH.

TABLE III

Carious, with care

NUMBER OF PATIENT	cc. $\frac{N}{200}$ HCl	cc. $\frac{N}{200}$ NaOH	NUMBER OF PATIENT	cc. $\frac{N}{200}$ HCl	cc. $\frac{N}{200}$ NaOH
4	18.40	4.80	21	15.36	2.74
	19.50	4.40	37	13.88	5.06
	17.30	3.65	45	7.20	7.42
	19.70	3.30	60	12.40	4.60
	11.44	5.50	71	2.95	1.70
	11.22	6.80	80	17.75	7.70
	13.00	5.58	81	28.80	5.82
	20.80	3.23	101	14.20	7.67
16	14.60	8.30	102	15.70	7.40
	14.00	12.90	104	17.30	17.40

TABLE IV

Carious, without care

NUMBER OF PATIENT	cc. $\frac{N}{200}$ HCl	cc. $\frac{N}{200}$ NaOH	NUMBER OF PATIENT	cc. $\frac{N}{200}$ HCl	cc. $\frac{N}{200}$ NaOH
6	16.60	7.10	39	7.20	9.22
	13.30	8.40	42	12.30	9.90
	11.00	7.50	47	8.16	3.10
9	4.70	9.70	52	16.32	1.00
	3.30	12.00	56	16.64	2.64
10	9.80	29.30	57	15.46	1.76
	4.20	23.30	62	7.90	12.70
15	13.38	3.12	69	11.50	4.53
	14.00	2.90	76	18.50	7.00
	16.50	2.20	77	17.45	15.00
17	9.40	8.10	82	8.95	18.33
18	2.80	22.10	84	28.90	3.70
	11.70	15.00	85	17.40	22.00
20	4.70	13.20	86	28.90	27.00
	12.90	5.18	87	24.20	2.76
	19.20	7.20	88	16.65	7.90
	20.90	5.00	90	19.95	10.00
	16.60	5.43	91	21.80	9.40
22	8.15	6.20	93	24.00	11.70
23	6.90	8.94	94	24.45	11.10
26	12.52	3.16	95	29.35	5.00
33	10.60	5.00	98	14.00	9.70
34	10.60	3.80	99	36.60	22.94
35	14.40	10.08	100	25.35	18.30
38	12.60	3.42			

From the above it is readily seen that no "normal" resting saliva exists for a variation of 16.20 cc., is rather too great to admit of establishing a factor which will definitely represent the "normal" condition of the saliva.

On further comparison also of tables 1 and 2, one observes that the looked for difference in clean or unclean mouths does not exist. That is, the acidities in resting saliva are relatively no higher in the one case than in the other. In table 2, patient 58, a notation in reference to the reaction to phenolphthalein is made. This man although an inmate of the institution for nearly four years is still unclassified. His physical condition is good and he is accorded the freedom of those who work outside. The writer can advance no theory for this exception in reaction.

TABLE V

Pyorrhea, with care

NUMBER OF PATIENT	cc. $\frac{N}{200}$ HCl	cc. $\frac{N}{200}$ NaOH
24	7.16	1.18
27	6.00	8.10

In noting the differences in acidity and alkalinity in tables 1, 2, 3 and 4, we fail to find any constant factor on which to base the assumption that the resting saliva in an unclean mouth has a higher acid or alkali content than the saliva from a clean mouth. In fact the condition of the saliva as resting, is too variable to establish a relationship between the acidity and alkalinity of a clean or unclean mouth, and the acidity and alkalinity of a carious or an immune mouth.

In tables 5 and 6 the number of cases presented is too small to attempt even an inference as to the relationship or lack of it that exists between pyorrhea and the reaction of saliva.

Tables 7 and 8 likewise show a lack of relationship. The high acidity as noted in table 7, patient 66, is noteworthy. In this particular case the form of the teeth invited decay. The embrasures between were not particularly well marked and the

TABLE VI

Pyorrhea, without care

NUMBER OF PATIENT	cc. $\frac{N}{200}$ HCl	cc. $\frac{N}{200}$ NaOH	NUMBER OF PATIENT	cc. $\frac{N}{200}$ HCl	cc. $\frac{N}{200}$ NaOH
31	9.84	6.40	83	21.55	7.00
43	15.14	4.80	84	28.90	3.70
62	7.90	12.70	85	17.40	22.00
82	8.95	18.33	86	28.90	27.00
73	9.75	15.60	87	24.20	2.76
72	29.10	3.44	90	19.95	10.00
	22.50	6.48	91	21.80	9.40

proximal contacts rather broad. The teeth were not of the bell crowned type but rather straight and flat.

All of the foregoing tables and above remarks have been in more or less opposition to the views held by the majority of the dental profession and may be said to be negative in character.

The following tables are, however, constructive in character, rather than destructive, and point to a physiological condition constantly present but which requires further study and experimentation.

It occurred to the writer that it might be interesting to note the stimulation reaction of saliva (using the same stimulus) in the mouths of those who have carious teeth and those who are not susceptible to caries. Chittenden and Richards (13) noted in 1898 "That mechanical stimulation, as chewing a tasteless substance, and alcohol, ether, gin, whisky taken into the mouth all lead to the outpouring of a saliva richer in alkaline reacting salts and amylolytic power, than the secretion coming *without* stimulation."

TABLE VII

Absolute immunity, with care

NUMBER OF PATIENT	cc. $\frac{N}{200}$ HCl	cc. $\frac{N}{200}$ NaOH
29	16.26	3.48
30	13.20	4.20
66	14.60	17.10

TABLE VIII

Absolute immunity, without care

NUMBER OF PATIENT	cc. $\frac{N}{200}$ HCl	cc. $\frac{N}{200}$ NaOH
25	10.38	7.20
28	15.40	3.32
44	17.00	2.80

It was along this line that the writer started the second series of experiments. The samples for these experiments were obtained as follows. A "normal resting saliva" was collected in the usual way. Then a small piece of white paraffine was given the individual with the instruction not to chew until the wax had become softened. When soft the chewing was commenced and continued until about 20 cc. of sample had been obtained. The time necessary for this was usually from fifteen to twenty minutes. The results are noted in tables 9 to 13 inclusive. In table 9 one notes in comparing the figures in column 3 with those in column 6 an astonishing drop in acidity, viz., from 17.10 cc. to 0.80 cc. and in comparing columns 2 and 5 a rise in alkalinity from 14.60 cc. to 43.02 cc. This interesting relation between

TABLE IX

Paraffine stimulus. Absolute immunity, with care

NUMBER OF PATIENT	NORMAL RESTING SALIVA			ACTIVATED SALIVA			
	cc. $\frac{N}{200}$ HCl	cc. $\frac{N}{200}$ NaOH	Neutralizing power	cc. $\frac{N}{200}$ HCl	cc. $\frac{N}{200}$ NaOH	Neutralizing power	Salivary factor
66	14.60	17.10	31.70	43.02	0.80	43.82	72.3

the drop of the acidity on the one hand and the rise of alkalinity on the other can best be shown in the following way.

In considering the reaction of the blood and some of the other tissue fluids Henderson (19), Robertson (20), and others, have shown that in measuring the power of the blood to maintain neutrality, this power may be estimated by *adding* the *acid* and *alkali titrations*; thus measuring the amount of reagent necessary to change the reaction of the blood from a definite alkalinity to a definite acidity.

In discussing this subject with Dr. Robertson he expressed the matter in the following words:

In seeking for a method of measuring the power of saliva to maintain itself neutral it is necessary to bear in mind the fact that no fluid can remain utterly unchanged in reaction as a result of adding acids or alkalies to it. Some change will ensue as a result of these additions

and that fluid possesses the greatest power of maintaining itself neutral or nearly neutral in which a given addition of acid or alkali produces the least change, or, conversely, in which a *given* change in acidity is brought about by the addition of the largest amount of reagent. We may measure the power of saliva to maintain its neutrality, therefore, in either of two ways—we may measure the amount of change produced by adding a given proportion of acid or alkali to the saliva—(the smaller the amount of change the greater the power of the saliva to maintain itself neutral) or, which is more convenient, we may measure the quantity of reagent required to change the saliva from one definite reaction to another definite reaction. The greater this quantity, the greater the power of the saliva to maintain itself neutral.

The reaction of normal resting saliva corresponds to no definite acidity or alkalinity—it varies not only from individual to individual but doubtless from moment to moment in the same individual, since the saliva is momentarily exposed to countless variable influences which must and do affect its precise reaction.

In order, therefore to measure the quantity of acid (or alkali) required to change the saliva from one given reaction to another given reaction we must first reduce all the samples of saliva to one definite condition (reaction) and, starting from this definite reaction estimate how much acid (or, reversely, alkali) must be added to this saliva to change it to another definite condition (reaction).

C
A...............|.......................B

Let "A B" be any quantity, for instance a distance or a volume (e.g., cubic centimeters of standard acid solution) which we desire to measure. If it be convenient, it is obvious that we can just as well measure "A B" by adding the distance "A C" to the distance "C B" as by measuring "A B" in one operation.

Applying this principle to the measurement of the power of saliva to maintain itself neutral, the position of "C" will represent the variable, fluctuating reaction of normal resting saliva. "A" will be a definite reaction on the acid side of neutrality (i.e., neutrality to para-nitrophenol) and "B" a definite reaction on the alkaline side of neutrality (i.e., neutrality to phenolphthalein). The distance "A B" is the number of cubic centimeters of acid required to change the saliva from reaction "B" to reaction "A" or, reversely, the number of cubic centimeters of alkali required to change the saliva from reaction "A" to reaction "B."

This distance is the sum of the distances "A C" and "C B," this is, of the number of cubic centimeters of acid required to bring the given sample of saliva to neutrality to para-nitro-phenol and the number of cubic centimeters of alkali which is required to bring the sample to neutrality to phenolphthalein. In other words *by adding together the results of the above titrations with acid and alkali we obtain a true measure of the power of the given samples of saliva to maintain their neutrality.* Neither of the titration values taken apart, affords such a measure, for, as explained above, the reaction of resting saliva (position of C in the above diagram) varies from moment to moment in consequence of events which we cannot estimate or define, hence the separate titres (distances

TABLE X

Paraffine stimulus. Present immunity, with care

NUMBER OF PATIENT	NORMAL RESTING SALIVA			ACTIVATED SALIVA			
	cc. $\frac{N}{100}$ HCl	cc. $\frac{N}{200}$ NaOH	Neutralizing power	cc. $\frac{N}{100}$ HCl	cc. $\frac{N}{200}$ NaOH	Neutralizing power	Salivary factor
1	20,20	6.42	26.62	37.82	2.10	39.92	66.00
2	12.90	8.20	21.10	47.50	1.70	49.20	43.00
3	14.70	3.40	18.10	37.40	1.70	39.10	46.00
67	13.70	4.97	18.67	34.95	1.13	36.08	51.70
70	17.90	7.78	25.68	49.45	2.50	51.95	49.40
70	14.45	5.10	19.55	43.80	1.20	45.00	43.60
74	19.80	8.00	27.80	45.35	1.90	47.25	58.80
75	26.60	4.30	30.90	50.36	1.93	52.29	59.10
89	24.25	2.70	26.95	32.80	1.40	34.20	75.90
103	7.80	7.53	15.33	14.65	3.90	18.55	82.65

A C or C B) varying from moment to moment in saliva of every individual *but their sum, "A B," remains, it is probable, relatively constant and characteristic.*

The samples for the following tables are from two classes of individuals. First, those persons who were regularly engaged in their daily routine as students, members of the office force, etc. Second, those persons, convalescent in the surgical wards of the different hospitals, who were taking no medication (except as noted) and who were on full diet.

Referring again to table 9 it is observed in this case of absolute immunity that the ratio, expressed in percentage, between the

neutralizing power of normal resting saliva and that of the activated saliva which the writer terms the "salivary factor," is 72.3.

Table 10 which is composed of cases of present immunity with care shows a relationship similar to the preceding table. The "salivary factor" with one exception is below 80, fluctuating between 43 and 75.9. Concerning the exception, patient Number 103, the writer will not attempt to theorize. The clinical record shows, however, an acid urine.

In table 11 the variation is nearly the same as in the two preceding tables and *all* percentages fall below 80. In patient

TABLE XI

Paraffine stimulus. Present immunity, without care

NUMBER OF PATIENT	NORMAL RESTING SALIVA			ACTIVATED SALIVA			
	cc. $\frac{N}{200}$ HCl	cc. $\frac{N}{200}$ NaOH	Neutralizing power	cc. $\frac{N}{200}$ HCl	cc. $\frac{N}{200}$ NaOH	Neutralizing power	Salivary factor
8	22.22	7.60	29.82	57.55	0.90	58.45	51.00
64	11.40	8.67	20.07	30.90	6.00	36.90	53.85
65	10.60	9.86	20.46	20.00	6.00	26.00	78.70
72	29.10	3.44	32.54	43.22	3.20	46.42	70.10
72	22.50	6.28	28.78	36.80	2.70	39.50	72.90
73	9.75	15.60	25.35	25.90	5.97	31.87	79.50
79	16.50	2.13	18.63	35.14	—0.40	35.14	53.00
83	21.55	7.00	28.55	39.20	4.00	43.20	66.10
96	28.80	6.97	35.77	56.20	0.70	56.90	62.90
97	25.60	4.45	30.05	37.70	1.40	39.10	76.90

79 the activated saliva was alkaline to phenolphthalein 0.40 cc. $\frac{N}{200}$ HCl. This was one of the very few exceptions to the general rule that saliva reacts acid to that indicator. Duplicate determinations were made on successive days the alkaline titration figure being 0.30 cc. $\frac{N}{200}$ HCl in one instance, and 0.70 cc. $\frac{N}{200}$ HCl in another. The salivary factor is constant within certain limits for the same individual when the same conditions obtain at the time each sample is taken. For instance, patient 72, varied on successive days between 70.1 and 72.9 per cent.

Turning now to table 12, "carious with care," a marked difference in the salivary factor is at once apparent; for in all

these cases it is above 80, fluctuating between 84 and 119.3. In this, and the following table, all degrees of carious conditions are presented.

Table 13, "carious without care," shows two exceptions. In the first case, Number 20, the patient was suffering from acute nephritis, hip arthritis, tuberculosis, and syphilis. These facts may, or may not, explain the low factor. In the second case, Number 94, the patient was taking sodium phosphate. Patient 93 is an example of an abnormally high salivary factor. The individual was suffering from peripheral neuritis and the disordered reflexes as evidenced in other ways might account

TABLE XII

Paraffine stimulus. Carious with care

NUMBER OF PATIENT	NORMAL RESTING SALIVA			ACTIVATED SALIVA			
	cc. $\frac{N}{200}$ HCl	cc. $\frac{N}{200}$ NaOH	Neutralizing power	cc. $\frac{N}{200}$ HCl	cc. $\frac{N}{200}$ NaOH	Neutralizing power	Salivary factor
4	20.80	3.23	24.03	26.40	2.00	28.40	84.6
80	17.75	7.70	25.45	24.40	2.52	26.92	94.5
81	28.80	5.82	34.62	32.74	2.22	34.96	99.0
101	14.20	7.67	21.87	15.05	5.50	20.55	106.4
102	15.70	7.40	23.10	19.45	4.00	23.45	98.5
104	17.30	17.40	24.70	25.80	3.30	29.10	119.3

for the high factor. In another case, patient 77, two sets of analyses are shown. The first set are from samples taken between 9.30 and 10.30 a.m. and the second from samples taken at 6 a.m. immediately on waking. The latter sample was not titrated until three hours after it had been collected and through error had been kept in a warm room in the interim. The writer suggests that these conditions might account for the difference in the two salivary factors for the same individual.

The following cases were all suffering from rampant caries:

Table 12, Patient 104.
Table 13, Patient 86.
Table 13, Patient 90.
Table 13, Patient 100.

It is interesting to note that in all these examples the salivary factor is abnormally high, being 119.3, 121.8, 132.8, 122.9, respectively. The number of cases, however, is so small that it would be presumptuous to infer that rampant caries is always associated with an exceptionally high salivary factor. It would appear, however, legitimate to conclude that in those persons susceptible to caries the ratio of the neutralizing power of the normal resting saliva compared to that of the activated saliva differs from the ratio observed in those immune from caries.

TABLE XIII

Paraffine stimulus. Carious without care

NUMBER OF PATIENT	NORMAL RESTING SALIVA			ACTIVATED SALIVA			
	cc. $\frac{N}{200}$ HCl	cc. $\frac{N}{200}$ NaOH	Neutralizing power	cc. $\frac{N}{200}$ HCl	cc. $\frac{N}{200}$ NaOH	Neutralizing power	Salivary factor
20	20.90	5.00	25.90	39.80	3.00	42.80	60.5
76	18.50	7.00	25.50	22.80	2.13	24.93	102.0
77	17.45	15.00	32.45	26.40	4.98	31.38	103.4
77 (6 a.m.)	24.75	22.00	46.75	32.92	6.00	38.92	120.0
82	8.95	18.33	27.28	24.35	4.30	28.65	95.0
84	28.90	3.70	32.60	30.50	0.50	31.00	105.0
85	17.40	22.00	39.40	31.95	4.63	36.58	107.7
86	28.90	27.00	55.90	40.50	6.40	46.90	121.8
87	24.20	2.76	26.96	26.75	2.00	28.75	93.8
90	19.95	10.00	29.95	20.15	2.47	22.62	132.8
91	21.80	9.40	31.20	30.50	3.70	34.20	91.2
93	24.00	11.70	35.70	19.50	5.73	25.23	160.6
94	24.45	11.10	35.55	41.00	9.40	50.40	70.5
95	29.35	5.00	34.35	30.75	4.00	34.75	98.9
98	14.00	9.70	23.70	22.86	2.00	24.86	95.3
99	36.60	22.94	59.54	45.40	12.60	58.00	102.6
100	25.35	18.30	43.85	32.55	2.97	35.52	122.9

Assume for example that the neutralizing power of the normal resting saliva is represented by 25 cc. and that of the activated saliva by 25 cc.; the ratio between the two is $\frac{25}{25}$ or unity or 100 per cent. Further, if the neutralizing power of the normal resting saliva is 20 and that of the activated saliva is 15 the ratio is $\frac{20}{15}$ or 133 per cent. In this latter case the normal resting saliva is the more potent of the two secretions in maintaining the neutrality of the oral cavity.

These two examples illustrate the ratio obtained in carious conditions. Consider now, the immune ratio. Assume that 15 cc. equals the neutralizing power of the normal resting saliva and that 25 cc. equals that of the activated saliva, then $\frac{15}{25}$ equals 60 per cent. In other words the power of the normal resting saliva is only 60 per cent of the power of the activated saliva and a reserve neutralizing power may be secreted under the necessary stimulus. In the carious condition, however, there is no reserve power or, at least, where the factor is between 84 and 100, it is too small to be of much effect; in fact in those cases where the factor is above 100 per cent the neutralizing power of the activated saliva is less than that of the normal resting saliva. If it can be determined what physiological process is concerned in the production of an activated saliva, containing, constantly, a greater neutralizing power than the normal resting saliva, the writer ventures to suggest that a definite step will be made in the elucidation of the etiology of dental caries.

Henderson (19), in considering the equilibrium between acids and bases in the animal organism says that "This acid-base equilibrium may stand in direct relation to bone formation, rickets and osteomalacia." What the relationship may be between this equilibrium in the saliva and dental caries is indicated in the above tables.

SUMMARY

1. That the reaction of normal resting saliva is too variable to be a positive factor in indicating any of the following conditions:

(a) Absolute immunity either with or without care.

(b) Present immunity either with or without care.

(c) Caries either with or without care.

2. That a method analogous to that employed by Henderson (19) in measuring the power of the blood to maintain neutrality is applicable to the determination of the same power shown by the activated saliva.

3. By means of this method a "salivary factor" may be evaluated, namely, the ratio of the neutralizing powers of the "normal

resting" and "activated" salivas respectively, the magnitude of which appears to be indicative of immunity from caries or the reverse.

4. In persons who are either absolutely immune or for the present immune from caries the magnitude of this factor (expressed in percentage) varies between 43 and 80, while in persons whose teeth are carious this factor varies between 80 and 132.

The writer avails himself of this opportunity to express his appreciation and to acknowledge his indebtedness to those members of the faculty and heads of institutions who have shown such a whole hearted interest in the work presented and who have made it possible for him to carry out these experiments. Without their coöperation it would have been exceedingly difficult to obtain patients and to work out the several lines of thought suggested.

BIBLIOGRAPHY

(1) HAMMARSTEN AND MANDEL: Textbook of Physiological Chemistry, 6th edition, 1911, New York, p. 343.
(2) JOHNSON'S Operative Dentistry, 1st edition, 1909, Philadelphia, p. 477.
(3) PICKERILL: Dental Caries and Oral Sepsis, 1st edition, 1912, London, p. 15, et. seq.
(4) GIES AND COLLABORATORS: Journal of Allied Societies, vol. 5, 1910, p. 262; vol. 6, 1911, p. 65 et. seq.; vol. 7, 1912, p. 199 et. seq.; vol. 8, 1913, p. 283.
(5) BUNTING: Dental Cosmos, March, 1914, p. 285.
(6) BÖDECKER: Dental Cosmos, June, 1914, p. 718.
(7) KIRK AND COLLABORATORS: Journal Allied Societies, vol. 9, June, 1914, p. 186.
(8) HOWE: Dental Cosmos, Nov., 1913, p. 1112.
(9) PAVLOV: The Work of the Digestive Glands, 2d Eng. edition, 1910, London, p. 68.
(10) COHN: Indicators and Test Papers, 2d edition, 1914, New York, p. 113, et. seq.
(11) SUTTON: Volumetric Analysis, 10th edition, 1911, Philadelphia, p. 38–44.
(12) RAYMOND: Human Physiology, 3d edition, 1905, Philadelphia, p. 181.
(13) CHITTENDEN AND RICHARDS: Journal of Physiology, 1, p. 476.
(14) ROSE: Journal Biol. Chem. 1, p. 2, 1911.
(15) THIERFELDER: Zeit. fur Physiol. Chem. 11, p. 388, 1887.
(16) TAYLOR: Digestion and Metabolism, Philadelphia, 1912, p. 120.
(17) STARLING: Human Physiology, Philadelphia, 1912, p. 740.

(18) ALLARIA: Monatschr. fur Kinderheilkunde, 10, 1911, p. 179.
(19) HENDERSON: Amer. Jour. Physiol. 1908, vol. 21, p. 169, et. seq.; 1906, vol. 15, p. 257; 1906, vol. 16, p. 188; 1907, vol. 18, p. 250; Ergeb. der Physiol., 1909, vol. 8, p. 254
(20) HALLIBURTON: Handbook of Physiology, 11th edition, Philadelphia, 1913, p. 509.
(21) ROBERTSON; Journal Biol. Chem. 7, no. 5, p. 351.
(22) TREADWELL-HALL: Analytical Chemistry, vol. 2, 3d edition, 1912, New York, p. 542.

THE INFLUENCE OF BLOOD TRANSFUSION ON THE HYPERGLYCEMIA AND GLYCOSURIA OF PANCREATIC DIABETES IN THE DOG

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In 1889 von Mering and Minkowski discovered that complete extirpation of the pancreas produces fatal diabetes.[1] The attempt of Pflüger to show that this diabetes following removal of the pancreas is due, not to the absence of the pancreas, but to injury to the duodenum and the nerves connecting the pancreas with the rest of the viscera, must be considered a failure.[2] The original conclusion of von Mering and Minkowski is definitely established: the loss of the pancreas results in fatal diabetes. But the subsequent endeavor to determine how absence of the pancreas causes diabetes is practically a record of repeated failures. The leading idea in all this work has been the internal secretion theory, or that the pancreas yields some substance to the blood in some way necessary for the oxidation of the sugar by the tissue cells. But in the absence of conclusive demonstration of the internal secretion the possibility that the work of the pancreas in maintaining normal sugar metabolism consists of detoxication processes must always be kept in view. The fact that even temporary glycosuria is not induced in normal animals by diabetic blood does not render the detoxication hypothesis untenable.

Some light has been thrown on the nature of pancreatic diabetes by results and experimental methods that may be inter-

[1] V. Mering and Minkowski: Arch. f. exp. Path. u. Pharm., 1889, xxvi, p. 371.

[2] Pflüger: Arch. f. d. ges. Physiologie, 1905, c. vi, p. 181; Minkoswki: Arch. f. exp. Path. u. Pharm., 1908, lix, p. 895.

preted either on the detoxication or the internal secretion theories. We refer to the methods of parabioses (Forschbach),[3] cross-circulation and transplantation (Hedon),[4] and pancreatectomy in late pregnancy (Carlson).[5] It is obvious that conditions which establish direct or indirect relation of the blood of a diabetic animal to living pancreas tissue may cause a change in the course of the disease either by detoxication or by internal secretion. On the other hand, if it can be shown that the oxidation of sugar by the tissues is augmented by some substance from the pancreas, or that pancreatic diabetes is temporarily ameliorated by transfusion of normal blood, the internal secretion theory is established.

The new method of attack introduced by Cohnheim[6] has not yielded consistent results, and in the light of the findings of Levene and Meyer the method itself is called in question, as it appears that in mixtures of sugar plus pancreas extract plus muscle extract, the sugar is polymerized, not oxidized.[7] The method of studying the action of pancreas extracts on the sugar consumption of the entire diabetic animal or on isolated but living organs of the diabetic animal is more physiological than that of Cohnheim, but the results to date are practically negative.[8] This may be due to failure to obtain the specific secretion in the pancreas extract, as well as to the difficulty of finding a reliable index of sugar oxidation in an isolated organ. In respect to this latter point it is obvious that our methods so far have been too crude or violent. The diabetic animal does not burn sugar, to any appreciable extent, which means that the individual organs of the diabetic animal do not burn sugar; yet when we compare the sugar consumption of the isolated organ of the

[3] Forschbach: Arch. f. exp. Path. u. Pharm., 1908, lx, p. 131.

[4] Hedon: Arch. intern. de. Phys., 1913, xiii, p. 4; p. 255.

[5] Carlson: Am. Jour. of Physiol., 1911, xxviii, p. 391; Jour. Biol. Ch., 1914, xvii, p. 19.

[6] Cohnheim: Zeitschr. f. physiol. Ch., 1903, xxxix, p. 336.

[7] Levene and Meyer: Jour. of Biol. Ch., 1911, ix, p. 389.

[8] Knowlton and Starling: Jour. of Physiol., 1912, xlv, p. 146; Maclean and Smedley: ibid., p. 420; Macleod and Pearce: Am. Jour. of Physiol., 1913, xxxii, p. 184; Patterson and Starling: Jour. of Physiol., 1913, xlvii, p. 137; Macleod and Pearce: Am. Jour. of Physiol., 1914, xxxiii, p. 378.

normal and the diabetic animal, we have so far failed to find any difference!

To our way of thinking the method least artificial and violent as regards the hypothetical pancreas secretion and the least injurious to the test object, that is, the diabetic animal, is the transfusion of normal blood. Yet, note the contradictory and ambiguous results from this mode of attack! Lepine reports a temporary diminution in the output of sugar in the urine, but no diminution in the blood sugar.[9] This would seem to point to some injurious action of the foreign blood on the kidneys. Hess injected intravenously 50-150 of blood from diabetic dogs into normal dogs (on the theory that diabetic blood might stimulate the pancreas to a greater output of the internal secretion) and 9–14 hours later he injected the serum from this animal into diabetic dogs. The influence on the glycosuria of the diabetic animal was slight or inconstant.[10] In view of the results reported from this laboratory a few years ago by Drennan,[11] it seems likely that in the experiments of Hess the pancreas secretion in the blood was destroyed by the delay in centrifuging the blood. Alexander and Ehrmann[12] injected blood from the pancreatic duodenal vein of normal dogs into diabetic dogs, but obtained no definite or constant decrease of the glycosuria. In some of the experiments secretin was added to the blood to be injected. The reason for this is not apparent. These investigators state that dogs with the pancreas completely extirpated but otherwise healthy and in good spirits sometimes void urine free from sugar, in the absence of all experimental interference. This has never been noted in this laboratory in the cases of more than 100 diabetic dogs, except in cases of incomplete pancreas extirpation.

Drennan, working in this laboratory, injected 50–150 cc. of fresh defibrinated dogs' blood into the veins of diabetic dogs and invariably obtained a temporary lowering of the urine sugar and

[9] Lepine: Le diobète sucré., 1909, p. 363.
[10] Hess: Münch. Med. Woch., 1902, p. 1449.
[11] Drennan: Am. Jour. of Physiol., 1911, xxviii, p. 396.
[12] Alexander and Ehrmann: Zeitschr. f. exp. Path., 1908, p. 367.

the D:N ratio. Defibrinated sterile blood loses this action on standing for a few hours. The course of the blood sugar in the injected animals was not studied. Hedon has reported a very extensive series of blood transfusion in diabetic dogs. Direct transfusion of normal blood into a diabetic dog previously bled dry causes a temporary lowering of the blood sugar and decrease or complete suppression of the glycosuria, but since the same results were produced when blood from a diabetic dog was transfused into another diabetic dog Hedon concludes that the temporary diminution of the hyperglycemia and glycosuria following the transfusion were not due to any specific pancreas secretion in the blood but to a lowering of the blood sugar by dilution and to a toxic action of the foreign blood on the kidneys. The results of the cross transfusion experiments reported by Hedon do not concern us here, since these may be interpreted in various ways (detoxication by the pancreas, storage of glycogen in the normal animal, dilution of the diabetic blood, etc.). Hedon also transfused (cross transfusion as well as serum injections) blood from the pancreatic vein of normal into diabetic dogs. A slight temporary lowering of the hyperglycemia with a greater reduction of the urine sugar was noted, but the latter is interpreted as due an injurious action of the foreign blood on the kidneys. Hedon concludes that the internal secretion of the pancreas acts on and is absorbed by the liver, and is therefore not present in the blood of the systemic circulation. Hedon attempted to obtain evidence in support of this view by introducing a living pancreas in the systemic and in the portal circulation of diabetic dogs. With the living pancreas interposed in the portal circulation the hyperglycemia and glycosuria were diminished, but interposed in the general circulation the pancreas had no effect. We do not think that these latter results of Hedon can be accepted, in view of what is known concerning the carbohydrate metabolism in dogs with Eck fistula. In the animal with the Eck fistula the internal secretion of the pancreas, if there is one, must pass into the general circulation, and only a small part of it can reach the liver by way of the hepatic artery, just as in Hedon's diabetic dogs with the living pancreas from

another dog interposed in the general circulation; yet the Eck fistula dog does not develop diabetes.

Murlin and Cramer[13] have recently reported one experiment with transfusion of normal blood into a diabetic dog, using the respiratory quotient as a measure of sugar oxidation. The average R.Q. for two one-hour periods before the transfusion was 0.678; for four one-hour periods after the transfusion 0.700. No conclusion can be based on the results of a single experiment, but so far as they go in this case, the transfusion raised the R.Q. Murlin and Cramer appear to have had in mind transfusion as "a measure of practical importance" in clinical diabetes, and are therefore disappointed with the results. To our way of thinking this one experiment was sufficiently encouraging to warrant ten or twenty additional ones, in the hope of settling the wexed question of internal pancreatic secretion, whether or not blood transfusion will be a measure of practical importance in clinical diabetes.

Raulston and Woodyatt appear to be the first to make use of blood transfusion as a practical therapeutic measure in diabetes melitus in man.[14] The patient was a man in the thirties, but the diabetes was of several years' standing, with severe acidosis, and periods of threatening coma. The blood (500 cc.) was yielded by a two year older brother of the patient. The sera of the two persons showed no lytic action on the respective corpuscles. *The blood transfusion augmented all the diabetic symptoms for several days.* In that respect this single transfusion test in clinical diabetes differs from all transfusion experiments in experimental diabetes so far in the literature. No investigator reports a single instance of blood transfusion aggrevating the pancreatic diabetes in dogs. In judging the importance of the negative results of this single test on man it must be taken into consideration that the patient was in the last stage of diabetes, and died in coma not long after the test. Furthermore, there is no evidence that this patient was primarily a case of pancreatic diabetes.

The above résumé goes to show that blood transfusion in

[13] Murlin and Cramer: Jour. of Biol. Ch., 1913, xv, p. 380.
[14] Raulston and Woodyatt: Jour. Am. Med. Ass., 1914, lxii, p. 996.

pancreatic diabetes has to date yielded positive (even if transitory), ambiguous or contradictory, and apparently negative results. This being the case, is there any justification for further attempts at analysis of the diabetes problem by the method of blood transfusion? We think there is and for the following reasons:

1. The results of transfusion experiments where the diabetic animal was bled dry or nearly dry before being transfused with normal blood are of doubtful value, because of the temporary asphyxia of the kidneys and the tissues in general by the excessive bleeding.

2. The results of prolonged cross transfusion or direct transfusion by means of direct connection of the blood vessels of donor and recipient are rendered practically valueless by use of anesthetics, or when anesthetics are not used, by the traumatism and struggling of donor and recipient.

3. Hedon's contention that the pancreatic internal secretion is found only in the portal circulation and that blood from the pancreas has no antidiabetic action unless introduced directly into the portal circulation is rendered untenable by the well known facts in the case of Eck fistula animals. It is probable that in Hedon's experiments the pancreas was too greatly traumatized or asphyxiated to function.

4. Negative results from injection of serum of normal animals into diabetic animals are of little or no significance, because the delay and manipulation of the blood to obtain the serum probably permits destruction by oxidation of most of the pancreatic secretion.

5. Extracts of the pancreas apparently injures the kidneys,[15] but Hedon's contention that transfusion of blood from one dog into another invariably injures the kidneys of the recipient in such a way that they become less permeable to sugar must be more firmly established, if it is to overthrow the positive results of transfusion in the way of decreased hyperglycemia and glycosuria.

[15] De Meyer: Arch. intern. de Physiol., 1909, viii, p. 121; Vahlen: Zeitsch. f. physiol. Ch., 1909, lix, p. 194.

6. Negative results of transfusion in advanced stages of absolute diabetes are probably of no significance as regards the question of an internal secretion of the pancreas, as in late diabetes secondary tissue changes are present, which the pancreas secretion is probably unable to modify directly.

EXPERIMENTAL PROCEDURE

The transfusion. In all of our experiments we aimed at transfusing a quantity of blood equal to about one-tenth the volume of the blood of the recipient. This was done without previous bleeding of the recipient. The blood was drawn from the carotid of the donor into a sterile syringe carefully oiled and injected into the recipient through a large hypodermic needle inserted into the saphenous vein. By working moderately fast there is no danger from coagulation. By this method the recipient is not subjected to anesthetics, traumatism, pain, or struggling. By transfusing only about one-tenth of the recipient's own blood volume there is no disturbance of the circulation from an excess of blood, and previous bleeding is therefore not necessary. It seems reasonable to suppose that the less disturbances the recipient is subjected to the more certain one can be in ascribing changes in the diabetes to some specific action of the transfused blood itself.

The determination of the blood sugar. The samples of blood for sugar determination were drawn from the saphenous vein by means of a hypodermic needle, or from the end of the tail. The quantity of blood drawn was determined by weighing. All the samples were approximately 5 cc. All our sugar determinations were made by the Rona-Michaelis' method, or a combination of this with the method of Bertrand. The tests were run in duplicates so far as this was possible.

The urine sugar was determined by the method of Benedict. The dogs were not catheterized as this cannot be done repeatedly without producing cystitis. Our 24-hour samples of urine are therefore subject to the time variations in voluntary micturition.

The care and feeding of the diabetic dogs. The pancreatectomized animals were fed on lean meat and water.

RESULTS

The influence of transfusion of normal blood on the hyperglycemia

The results of our 20 experiments on 12 diabetic dogs are given in Table I. In no case did the transfusion increase the diabetic hyperglycemia. In three experiments (Dog I, expt. 3, and Dog II, expt. 1; Dog V, expt. 1). There appears to be no change in the blood sugar of the recipient. In all the remaining experiments there is a lowering of the hyperglycemia, but in two cases (Dog II, expts. 2 and 3) is too small to have any significance. The decrease in the hyperglycemia is very temporary, probably not exceeding 6–10 hours.

The experiments on Dogs VII–X demonstrate that this low ering of the hyperglycemia is not a matter of dilution of the diabetic with the normal blood, as the dilution factor must be at its maximum within a few minutes after completion of the transfusion. That dilution is not an important factor can be shown in other ways. For example, in a diabetic dog having 750 cc. of blood with a sugar content of 0.25 per cent transfusing 75 cc. of blood from a normal dog with the blood sugar 0.10 per cent can by dilution alone reduce the hyperglycemia from 0.25 per cent to 0.238 per cent. In this connection we may also note the results of three experiments in which we made intravenous injections of Ringer's solution in amounts equal to one-tenth of the total quantity of blood in the diabetic dog:

Experiment 1. Blood sugar before transfusion 0.34; blood sugar 3½ hrs; after transfusion 0.30.

Experiment 2. Blood sugar before transfusion 0.25; blood sugar 2½ hrs; after transfusion 0.25.

Experiment 3. Blood sugar before transfusion 0.25; blood sugar 2¼ hrs; after transfusion 0.26.

Since the output of sugar in the urine in diabetes is proportional to the hyperglycemia, it is evident that the temporary reduction of the hyperglycemia of pancreatic diabetes by transfusion of normal blood is a factor in the parallel reduction of the glycosuria.

TABLE I.

The influence on the hyperglycemia of pancreatic diabetes in dogs of transfusion of blood from normal dogs. The blood sugar of the donors varied from 0.07 per cent to 0.10 per cent. The last column gives the time after the transfusion when the samples of blood were drawn.

DOG	EXPERIMENT	BLOOD SUGAR		TIME AFTER TRANSFUSION
		Before trans.	After trans.	
I	1	0.23	0.15	2½ hrs.
	2	0.23	0.14	1¾ hrs.
	3	0.17	0.17	2½ hrs.
II	1*	0.12	0.12	2½ hrs.
	2	0.21	0.19	5 hrs.
	3	0.33	0.30	5 min.
			0.32	2 hrs.
III	1	0.32	0.15	3 hrs.
	2	0.30	0.26	4½ hrs.
IV	1	0.23	0.15	5½ hrs.
	2	0.20	0.17	1¾ hrs.
	3	0.19	0.16	4⅓ hrs.
V	1†	0.17	0.17	4¾ hrs.
	2	0.25	0.22	10 min.
			0.20	2⅓ hrs.
VI	1	0.26	0.16	10 min.
			0.19	1¾ hrs.
VII	1	0.25	0.20	5 min.
			0.12	1 hr.
			0.17	2½ hrs.
			0.28	24 hrs.
VIII	1	0.29	0.25	5 min.
			0.21	1⅙ hrs.
			0.15	3¾ hrs.
IX	1	0.29	0.25	5 min.
			0.21	1 hr.
			0.25	2⅓ hrs.
X	1	0.25	0.23	6 min.
			0.19	1¼ hrs.
			0.19	4 hrs.
			0.23	24 hrs.
XI	1	0.29	0.26	5 min.
			0.27	2¼ hrs.
XII	1	0.35	0.28	5 min.
			0.26	2⅓ hrs.

* Sandmeyer type of diabetes owing to trace of pancreas left on duodenum.

† In this experiment the donor was an old dog with adenomatous cancer of the thyroid.

The influence of the transfusion of normal blood on the glycosuria of pancreatic diabetes

The results of our 16 experiments on 9 dogs are summarized in Table II. The data in this table seem to warrant the following conclusions:

1. The glycosuria is in no case augmented by the transfusion.

TABLE II.

The influence of transfusion of normal blood on the glycosuria of pancreatic diabetes in dogs. The 24 hours volume of urine is only approximate, as the dogs were not catheterized.

DOG	EXPERIMENT	24 HOURS VOLUME OF URINE			24 HOURS OUTPUT OF URINE SUGAR		
		Day before transfusion	Day of transfusion	Day following transfusion	Day before transfusion	Day of transfusion	Day following transfusion
		cc.	*cc.*	*cc.*	*gr.*	*gr.*	*gr.*
	1	640	560	510	20.50	12.300	17.34
I	2	680	660	680	21.48	14.570	19.65
II		380	187	160	10.00	2.390	7.50
III	1	234	176	220	16.38	6.840	17.80
	2	173	107	250	14.60	3.780	16.85
IV	1	500	630	450	10.20	5.300	10.26
	2	450	195	500	12.40	2.140	10.10
V		185	160	170	3.60	1.150	3.95
VI	1	250	220	204	16.42	3.760	10.62
	2	160	130	170	8.96	0.557	3.74
VII	1	650	560	490	18.98	5.000	17.53
	2	485	450	400	15.37	3.200	10.00
VIII	1	625	980	860	22.80	6.300	28.90
	2	550	653	655	16.42	7.500	16.60
IX	1	540	377	376	13.00	5.440	6.68
	2	353	261	540	10.57	3.900	13.10

2. The transfusion diminishes the glycosuria, but in no case is the urine rendered sugar free.

3. In every case when frequent samples of urine were obtained after the transfusion the lowering of the urine sugar lasted only 4–10 hours after the transfusion, that is to say, the depression of the glycosuria runs parallel with the lowering of the hyperglycemia.

4. In most cases there is some depression of the polyuria parallel

with the decreased sugar output, but the effect of the transfusion appears most strikingly in the percentage of the urine sugar.

5. There is no evidence that the sugar retained by the dogs for the first 24-hour period following the transfusion is eliminated as excess sugar during the subsequent 24 hours. The reader may note that there is a slight indication of such a possibility in experiment 1, Dog VIII, but in some of the other experiments the relations are exactly reversed. These small fluctuations in either direction are therefore of no significance.

The influence of transfusion of diabetic blood on the hyperglycemia of pancreatic diabetes

The reader will recall that according to Hedon transfusion as such produces some temporary injury to the kidneys in consequence of which the output of sugar in the urine is temporarily diminished. Hedon reports the same results from transfusion of diabetic blood as from transfusion of normal blood. With our method of transfusion we have been unable to confirm Hedon on this point. Our ten experiments on six dogs are summarized in Table III. The transfusion (according to our method) of blood from a dog in complete pancreatic diabetes has no effect on the hyperglycemia of the latter. This fact indicates that the

TABLE III.

Transfusion of blood from dogs in pancreatic diabetes into dogs in pancreatic diabetes. The transfusion does not influence the hyperglycemia of the recipient.

DOG	BEFORE TRANSFUSION	AFTER TRANSFUSION	TIME
			hours
I*	0.120	0.120	3
II	0.210	0.210	2
	0.260	0.270	2
	0.280	0.280	2
III	0.240	0.235	2
	0.237	0.267	2
IV	0.310	0.308	2
	0.270	0.255	2½
V	0.210	0.220	1
VI	0.300	0.290	3

* Dog with incomplete diabetes (Sandmeyer type).

lowering of the hyperglycemia of pancreatic diabetes by the transfusion of normal blood is not due to depression of the tissues in general and hence lowering of the sugar production.

The influence of transfusion of normal blood into normal dogs on the sugar tolerance of the recipient

The suggestion of Hedon that blood transfusion as such injures the kidneys in such a way as to temporarily diminish sugar excretion is so important for the interpretation of all results obtained by transfusion methods that it should be tested in all possible ways before it is finally rejected, or accepted. The following tests on a series of normal animals were accordingly carried out. Seventeen dogs were injected intravenously with 1.5 gram dextrose (20 per cent solution) per kilo body weight and the quantity of sugar excreted during the following 24-hour period determined. Another series of nineteen dogs were transfused with normal blood by the same method employed in our diabetic dogs before receiving corresponding quantities of dextrose intravenously. If this blood transfusion injures the kidneys in such a way as to temporarily decrease their capacity to excrete sugar we would expect less sugar in the urine of these dogs, as a normal dog is capable of storing and burning large quantities of sugar. Our results did not come out in that way. *The average total output of sugar in the urine was 0.96 gram for the control group and 1.33 gram for the transfused group.* It is well known that there is considerable individual variations in the sugar tolerance in dogs, whether tested by the hypodermic, the intramuscular, or the intravenous methods, and both of our series showed these individual fluctuations. We therefore deemed it necessary to use the above great number of dogs in order to neutralize this source of error as far as possible.

In normal dogs and with normal blood, our method of transfusion does not impair the power of the kidneys to excrete sugar during hyperglycemia. So far as the transfusion has any effect at all it appears to increase the sugar excretion. But the final establishment and explanation of this latter point requires further investigation.

The influence of blood transfusion on normal and diabetic dogs on the general secretory activity of the kidneys

It seemed to us, that if blood transfusion as such injures the kidneys sufficiently to depress their power to excrete sugar, such injury ought to appear in the capacity of the kidneys to excrete some or all of the normal constituents of the urine. The work on this phase of the general question was done in our laboratory by Mr. I. Rabens, and the detailed results will be reported later. In normal and in pancreatectomized dogs on standard rations of lean meat and fixed quantities of water, the total nitrogen, urea, ammonia, amino acids, phosphorus, chlorides and (in the diabetic dogs) sugar were determined for 24-hour periods before and after transfusion with normal blood. In the normal dogs the transfusion had no effect on the kidney activity; in the diabetic dogs the transfusion diminished only one of the above constituents, namely the sugar.

It would therefore seem that our method of blood transfusion has no demonstrable injurious action on the kidneys either in normal or in diabetic dogs. Hedon's explanation of the effect of blood transfusion on the glycosuria and hyperglycemia of pancreatic diabetes is therefore untenable, at least as regards our results.

DISCUSSION OF THE RESULTS

The transfusion of normal blood into dogs in pancreatic diabetes causes a temporary lowering of the hyperglycemia. But before this can be taken as an evidence of the presence in normal blood of an internal secretion from the pancreas, it must be shown that this temporary lowering of the hyperglycemia is due to storage and oxidation of sugar in the tissues. Our work indicates that sugar retained as a result of the blood transfusion is not subsequently excreted. If this is the case what can happen to this sugar except oxidation in the tissues? In the first place, a temporary lowering of the rate of tissue metabolism might account for the temporary fall in blood sugar. Or, the metabolism rate being the same, some of the sugar may disappear by

way of the digestive secretions[16] and bacterial oxidation in the digestive tract. However improbable these possibilities may seem, they must be reckoned with in the absence of definite proof that this sugar is oxidized by the diabetic tissues.

SUMMARY

The transfusion of normal blood into dogs in complete pancreatic diabetes, by the method outlined above, causes a temporary (4–8 hours) lowering of the hyperglycemia and the glycosuria.

Similar transfusions of diabetic blood into diabetic dogs have no effect on the hyperglycemia.

There is no indication in our results that the sugar retained by the animal in consequence of this temporary lowering of the sugar excretion by the kidneys is subsequently eliminated by the kidneys as excess sugar.

The blood transfusion as such does not impair the kidneys' activity in any demonstrable way, either in diabetic or in normal dogs. The temporary lowering of the glycosuria of pancreatic diabetes by transfusion of normal blood is due to the diminished hyperglycemia, not to kidney injury.

[16] Carlson and Ryan: Am. Jour. of Physiol., 1908, xxi, p. 301.

THE INFLUENCE OF BLOOD TRANSFUSION ON THE KIDNEYS

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Hedon[1] reports that transfusion of normal as well as diabetic blood into diabetic dogs leads to a temporary decrease in the glycosuria. According to Hedon the foreign blood received by the diabetic animal is in some way injurious to the kidney, and the lowered glycosuria following a transfusion is not due to introducing blood containing the internal secretion which would temporarily help the tissues to utilize the sugar in the blood, but to a decreased permeability of the kidney to sugars through the injurious action of the foreign blood, normal or diabetic.

Carlson and Ginsburg[2] found a lowered glycemia accompanying the decreased glycosuria after transfusion of normal blood into diabetic dogs. The reduced sugar content of the urine could thus be accounted for by the reduced sugar content of the blood without any injurious action on the kidneys as claimed by Hedon. Moreover, they found no change in the glycemia or glycosuria following transfusions with diabetic blood. The fact that Hedon obtained a reduction in the glycosuria after transfusion with diabetic blood they believed to be due to his severe method of transfusion. They also tested the sugar tolerance of normal dogs before and after transfusion with normal blood, and found no increased tolerance after transfusion as would be expected if the foreign blood produced a decreased permeability of the kidneys to sugar.

At the suggestion of Dr. Carlson the following work was undertaken as an additional test of the possible injurious action of

[1] Hedon, Arch. internat. de physiol., 1913, xiii, p. 4, p. 255.
[2] Carlson and Ginsburg, This Journal, 1915, xxxvi, p. 280.

transfusion on the kidneys in normal and in diabetic dogs. It would seem that if blood transfusion had any direct effect on the kidneys this should be disclosed by a quantitative determination of the principal urinary constituents eliminated for 24-hour periods by both the normal and diabetic animal before and after blood transfusion.

This was the primary object of the work. Indirectly the results of these tests may also throw light on the effects on the kidneys of blood transfusion in man. Transfusion is coming into greater vogue clinically, especially in the spontaneous infantile hemorrhages, in anemias, in shock, where at least a temporary rise of blood pressure is looked for, and in septicemias, where an increased supply of antibodies is desired.

EXPERIMENTAL PROCEDURE

The dogs were kept in ordinary metabolism cages, and fed daily on a definite quantity of boiled fresh beef-heart freed from fat as completely as possible. They were also given a definite quantity of water, once daily, and generally by a stomach tube.

The transfusions were made for me by Dr. Carlson and Mr. Ginsburg according to the method employed by them in the previous work. A fairly large young dog was selected to serve as donor. This was done to provide against the possibility of a spontaneous nephritis often observed in old dogs in this region.[3] The amount of blood injected was calculated to approximate roughly one-seventh to one-tenth of the entire blood content of the recipient.

The quantitative determinations were made of the following urinary constituents. The determinations were made in duplicate to guard against any possible error in technique.

1. *Total nitrogen* by the Kjeldahl method as modified by Gunning and Arnold.

2. *Total urea* by the Marshal urease method (modified by F. C. Koch).

3. NH_3 by the Folin aeration method (modified by F. C. Koch).

[3] Personal communication from Dr. C. E. King.

4. *Amino acids* estimation by the Formol Titration.
5. *Total phosphorus* by the Neumann-Pemberton Method.
6. *Total chlorides* by the modified Volhard Method.
7. *Reducing sugars* by the Munson-Walker and Bertrand method.
8. *Total acidity and specific gravity.*

In the diabetic dogs the urine sugar was determined on every sample of urine voided the first 24 hours following the transfusion. The estimation of the other constituents were made on the 24-hour sample. In the calculations both the total and percentage of each constituent were figured out, for the volume of urine varied somewhat from day to day. Eight dogs were used in this work, 10 transfusions being made on normal, and 5 on diabetic animals.

RESULTS

Our results fail to bring out any evidence of injury or depression of the kidneys by Carlson's method of transfusion, as will be seen by examination of the three typical sets of experiments summarized in Tables I to III. There are some variations in all the urine constituents from day to day, but with the exception

TABLE I

Normal dog No. II. Weight, 10 kgm.; receiving 300 grams boiled beef heart daily. Analysis of the urine for three days before (control) and three days following transfusion of 75 cc. of blood from another dog

	3 DAYS CONTROL PERIOD			3 DAYS TRANSFUSION PERIOD		
	1	2	3	1	2	3
Volume of urine, cc.	325.	300.	325.	350.	400.	290.
Sh. sp. gr.	1034.	1026.	1025.	1026.	1021.	1030.
Acidity (in cc. of $\frac{N}{10}$ NaOH per 100 cc.)	97.5	68.0	61.2	81.0	54.50	74.8
Total nitrogen, grams	10.000	7.560	7.190	8.800	8.230	9.110
Amino acid nitrogen, grams	0.406	0.353	0.375	0.376	0.336	0.461
Urea, grams	10.450	14.040	10.350	11.390	11.060	7.680
Ammonia, grams	0.371	0.365	0.456	0.497	0.443	0.507
Chlorides, grams	0.247	0.213	0.345	0.423	0.487	0.494
Phosphorus, grams	0.800	0.610	0.610	0.720	0.680	0.740

TABLE II

Dog VI. Weight, 8 kgm. Dog in complete pancreatic diabetes. Analysis of urine for 24 hours before and for two days following transfusion of 100 cc. blood from a normal dog. Dog fed 200 grams boiled beef heart daily

	BEFORE TRANSFUSION 24 HOURS	AFTER TRANSFUSION	
		1st day	2d day
Volume of urine	350.	400.	300.
Specific gravity	1044.	1024.	1043.
Acidity (in cc. of 0.1 NaOH per 100 cc.)	80.	46.	68.
Total nitrogen, grams	8.040	7.840	6.000
Amino acid nitrogen, grams	0.580	0.480	0.510
Urea, grams	7.240	5.820	5.220
Ammonia, grams	0.530	0.560	0.510
Chlorides, grams	0.015	0.025	0.011
Phosphorus, grams	0.430	0.500	0.480
Sugar, grams	19.180	10.520	17.480

TABLE III

Dog V. Weight, 9.7 kgm. Dog in complete pancreatic diabetes. Analysis of the urine for 24 hours before and for two days following transfusion of 100 cc. of blood from a normal dog. Dog given 200 grams boiled beef heart daily

	BEFORE TRANSFUSION 24 HOURS	AFTER TRANSFUSION	
		1st day	2d day
Volume of urine	350.	320.	350.
Specific gravity	1043.	1033.	1046.
Acidity	57.	41.	60.
Total nitrogen, grams	6.930	5.220	7.060
Amino acid nitrogen, grams	0.562	0.466	0.592
Urea, grams	8.210	4.250	6.360
Ammonia, grams	0.600	0.550	0.710
Chlorides, grams	0.017	0.091	0.025
Phosphorus, grams	0.520	0.470	0.650
Sugar, grams	17.500	14.000	16.500

of the urine sugar in the case of the diabetic dogs, these daily variations are not definitely related to the transfusion. There are also fluctuations in the percentage of the urine sugar in the diabetic dogs, but these do not account for the characteristic lowering of the glycosuria by the transfusion (Table IV). In 1 out of the 5 transfusion experiments in the diabetic dogs, there is a slight diminution in practically all of the urine constituents

for the first 24 hours following the transfusion (see Table III). The other four groups of the diabetic dogs are practically identical with the one given in Table II. It is therefore clear that the temporary decrease in the glycosuria following these transfusions cannot be accounted for by kidney injury. So far as can be determined by examination of the urine this transfusion has no effect on the kidneys or on the general level of the body metabolism.

TABLE IV

Diabetic dog V. Sugar determinations of every sample of urine voided for 24 hours before and for 24 hours after transfusion of dog VI (see Table II)

BEFORE TRANSFUSION			AFTER TRANSFUSION		
Time	Sugar	Sugar	Time	Sugar	Sugar
hours	*grams*	*per cent*	*hours*	*grams*	*per cent*
23	2.72	5.33	2	2.20	2.50
20	2.42	5.50	3½	1.48	2.64
17	2.59	6.47	5¾	1.40	1.91
12½	1.75	5.73	9½	1.15	1.79
2	7.85	5.49	12	2.04	4.16
			21	2.64	3.38
Total..........	19.18	5.48	Total.....	10.52	2.65

The reader will note the very low output of total chlorides in diabetic dogs. This was a uniform result. On the same diet the diabetic dogs eliminated in the urine only about one-seventh to about one-tenth of the chlorides found in the urine before pancreatectomy. Are these chlorides eliminated in the feces? Or, are they retained in the tissues? These dogs showed no evidence of edema, and Dr. Carlson informs me that he has never observed any tendency to edema in pancreatic diabetes in dogs. The cause of this lowered output of urine chlorides in pancreatic diabetes is being further investigated in this laboratory.

We desire to express our hearty gratitude to Drs. A. J. Carlson and F. C. Koch for their supervision and advice while this work was in process.

THE THRESHOLD STIMULUS OF THE CHORDA TYMPANI NERVE IN RELATION TO SALIVARY SECRETION AND VASODILATION

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A vast amount of work has been done on the chorda tympani nerve in regard to secretion and vasodilation, but no accurate determination of the threshold stimulus of this nerve as far as I am aware has yet been made. In some cases the investigators attempted to obtain some idea of how strong a current is necessary to produce a secretion of saliva or to bring about dilation of the vessels in the submaxillary gland supplied by this nerve. They employed the method of moving the secondary coil a certain distance from the primary coil without taking into consideration tissue resistance. Since it is only recently that a reliable method for determining the strength of faradic stimuli has been worked out, it is not surprising to find very little literature directly pertaining to the subject here involved.

The present investigation was undertaken (1) to determine what is the weakest stimulus required by the chorda tympani (a) to produce a secretion of saliva, (b) to produce dilation of the vessels supplied by this nerve and (2) to determine whether or not a difference exists in the threshold stimuli necessary to produce these phenomena.

THE METHOD

Dogs, anaesthetized with ether and quickly decerebrated, were employed in these experiments.[1] The skin was cut on the median line of the neck and the mylo-hyoid muscle cut and laid

[1] Gruber: This Journal, 1913, xxxii, p. 438.

back exposing the chorda tympani nerve and the submaxillary duct. The nerve was isolated and freed from as much fascia as possible. A glass electrode[2] was placed on the nerve so that the cathode was proximal to the gland. Stretching and unnecessary handling of the nerve were avoided.

The method used to indicate the presence of salivary secretion was very simple. A cannula was inserted in Wharton's duct. As the saliva was secreted it fell in drops upon a lever attached to a receiving tambour, which, in turn, was connected to a recording tambour. When very weak currents—those nearing the threshold—were used direct observation of the saliva accumulating at the end of the cannula was necessary.

In much the same manner vasodilation was recorded. A cannula was placed in the external jugular vein after all the veins contributing to it, save the one supplying the submaxillary gland, had been tied off. The rate of blood flow was recorded in drops. Each drop, falling on a lever attached to an electro-magnetic signal, broke the circuit, thus causing a mark to be made on the surface of the drum by the signal. Later tambours were used instead—a receiving and a recording tambour—like those used in recording salivary secretion.

The threshold stimulus was measured by means of the Martin method in which the strength of stimulus is calculated in β units.[3] The position of the secondary coil, in every case, was read by moving it away from the primary coil until the smallest amount of vasodilation or of secretion of saliva occurred. Four of these readings were made, one with tissue resistance and the others with 10,000, 20,000 and 30,000 ohms resistance in the secondary circuit. Immediately after the determination of the position of the secondary coil, before the electrodes were disturbed in any way, three readings of tissue resistance were made.[4]

The strength of the primary current for determining the threshold was either 0.1 or 0.2 amperes. The rate of stimulation was usually five per second. This rate was chosen because it was

[2] Sherrington: Journal of Physiology, 1909, xxxviii, p. 382.
[3] Martin: Measurement of Induction Shocks, New York, 1912, pp. 60–69.
[4] Martin: *Loc. cit.*, pp. 71–93.

found favorable to produce dilation of the vessels supplying the extremities,[5] it seemed therefore probable that it might prove favorable in the production of dilation of the vessels supplied by the chorda tympani. Slower and more rapid rates were also employed. The inductorium which was used throughout had a secondary resistance of 2400 ohms. This was added to the average tissue resistance in making corrections. Corrections were also made for core magnetization. In this coil the value of K was 0.22.[6]

Carlson found it possible upon stimulating the cervical sympathetic to obtain, in some cats, marked dilation of the vessels without secretion of saliva.[7] Such was not the case, I found, upon stimulation of the chorda tympani. In the experiments here performed the strength of stimulus necessary to produce secretion also produce vasodilation in every case and vice versa. Table 1 shows the thresholds thus obtained. The Z units varied from 2.2 to 12.80 or an average for the sixteen experiments of 5.4 Z units. β units were calculated each time and these varied from 1.6 to 7.58 or an average for the sixteen experiments of 3.14 β units (see table 1).

The results here obtained are interesting in that they agree closely with those obtained upon stimulation of the cervical sympathetic in cats. Mendenhall found the threshold of this nerve for constriction of the vessels of the nasal membrane to be 7.89 Z units and 4.58 β units.[8] Still more striking is the similarity between the threshold of the chorda tympani and the threshold of the cervical sympathetic when the average of the thresholds (pupil, nictating membrane, and nasal vasoconstrictors) of the latter nerve is taken—6.65 Z units and 3.86 β units. This similarity can easily be understood if we consider the fact that a ganglion is traversed in each case. Langley has shown the presence of nerve cells within the hilus of the submaxillary gland

[5] Bowditch and Warren: Journal of Physiology, 1886, vii, p. 416; Bradford: *ibid.*, 1889, x, p. 390.

[6] Martin: *Loc. cit.*, p. 46.

[7] Carlson: This Journal, 1907, xix, p. 409.

[8] Mendenhall: This Journal, 1914, xxxvi, p. 60.

itself,[9] which cells bear the same relation to the chorda tympani as does the superior cervical sympathetic ganglion to the cervical sympathetic nerve.

It can be seen, upon comparison, that the thresholds of the chorda tympani and the flexion reflex of the hind leg of a cat, are markedly similar. Porter found 5.2 Z units and 2.7 β units necessary to arouse this reflex.[10] In my experiments on the

TABLE I

The threshold stimulus of the chorda tympani nerve as shown by the dilation of the vessels in the submaxillary gland and by the secretion of saliva

Z UNITS	β UNITS	RATIO OF β TO Z
2.20	1.60	0.73
3.65	1.83	0.50
3.65	1.60	0.44
3.65	3.20	0.90
4.17	2.06	0.50
4.17	2.40	0.58
4.20	2.40	0.57
4.38	1.74	0.39
4.59	2.03	0.43
4.59	2.90	0.63
4.59	2.86	0.62
4.59	3.26	0.71
5.22	2.20	0.42
6.37	4.44	0.70
7.94	2.96	0.37
11.90	8.80	0.74
12.80	7.58	0.59
Average.... 5.45	3.14	0.58

peroneus communis nerve and the tibialis anticus muscle (nerve muscle preparation) I found that the threshold stimulus was 1.2 β units.[11] Porter found the threshold for a simple nerve muscle to be 1.4 β units.

For thirty-one experiments Porter obtained a ratio of β to Z of 0.57.[12] Mendenhall found the ratio for his twelve experi-

[9] Langley: Journal of Physiology, 1890, xi, p. 125.
[10] Porter: This Journal, 1912, xxxi, p. 148.
[11] Gruber: *Loc. cit.*, p. 477.
[12] Porter: *Loc. cit.*, p. 148.

ments to be 0.58.[13] In these sixteen experiments the ratio for the average β to Z is 0.58. This, however, is merely a coincidence because if any experiment is omitted from the series, the ratio is changed. For example if the last is omitted then the ratio is 0.52 instead of 0.58. It has been suggested recently that this ratio may be taken as a quick means of calculating β units for a whole series of experiments. Upon this suggestion I reviewed my experiments upon the thresholds of the peroneus communis nerve and the tibialis anticus muscle. It may be well to give the

TABLE II

The average thresholds obtained upon the peroneus communis nerve and the tibalis anticus muscle

	AVERAGE Z	AVERAGE β	RATIO OF AVERAGE β TO Z	NUMBER OF EXPERIMENTS
Nerve muscle	1.78*	1.18	0.63	16
	3.90*	3.08	0.78	10
	1.33†	0.80	0.60	8
Muscle directly	25.82*	18.80	0.73	15
	40.26*	29.85	0.74	10
	23.50†	18.70	0.79	8
	28.40‡	25.00	0.74	8
Denervated muscle	150.50‡	61.40	0.40	6
Chorda tympani	5.45	3.14	0.58	17

* Gruber: This Journal, 1913, xxxii, p. 477; *ibid*, p. 448.
† *Ibid*, 1914, xxxiii, p. 338.
‡ *Ibid*, 1914, xxxiv, p. 92.

average Z units and the average β units of those experiments in this connection (see table 2).

From table 2 it can be seen that a wide variation exists in the ratios of β to Z in the several series of experiments performed. If however, we take the ratio of the average β to the average Z it will be 0.57. But if an average of the ratios is made it will be 0.66. Moreover, since the value of β depends upon the tissue resistance as well as upon the Z units it is quite important that the tissue resistance in all cases be the same if β is to be calculated

[13] Mendenhall: *Loc. cit.*, p. 60.

in this way. In table 1 we have three examples which show variations in β's calculated from like Z's with different tissue resistances, e.g., two Z values of 4.17 with resulting β units of 2.0 and 2.4, two Z values of 3.65 with β units of 3.20 and 1.6, and three Z units of 4.59 with β units of 2.03, 3.26 and 2.9. Porter shows a marked difference between ratios of single β's and their Z's. These range from 0.23 to 0.92 with an average ratio of 0.59 for the seventeen experiments on the flexion reflex and from 0.13 to 0.83 with an average ratio of 0.55 for the fourteen experiments on the nerve muscle.[14] From these results it is quite evident that the Martin formula or the formula established by Wilbur must be employed to calculate the β units accurately even for a long series of experiments.[15]

A few experiments were performed by employing different rates of stimulation. The threshold stimulus remained the same when the rate was from 3 to 15 per second. As the rate was decreased below 3 per second the threshold increased. It was possible to bring about a secretion by a single make or break shock but the strength of current necessary was greatly in excess of the threshold stimulus.

SUMMARY

1. The threshold stimulus for dilation of the vessels in the submaxillary gland and the secretion of saliva of this gland are the same.

2. The rate of stimulation within certain limits (3 to 15 per second) does not influence the thresholds of vasodilation and salivary secretion. For lower rates an increase in the threshold stimulus is necessary to bring about these changes.

3. The average threshold stimulus of the chorda tympani was found to be 5.4 Z units and 3.14 β units. This threshold is practically the same as that found by Mendenhall for the cervical sympathetic nerve.

4. A fixed ratio of β to Z cannot be used to determine accurately the β units of a series of experiments of any length.

[14] Porter: *Loc. cit.*, p. 148.

[15] Martin: *Loc. cit.*, p. 60; Martin Bigelow and Wilbur: This Journal, 1914, xxxiii, p. 416.

FACTORS AFFECTING THE COAGULATION TIME OF BLOOD

VI. The Effect of Rapid Progressive Hemorrhage upon the Factors of Coagulation

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It is an old observation that progressive bleeding decreases the coagulation time of the blood. Gray and Lunt[1] have summarized the literature on this subject. We will, however, review briefly the chief explanations which have been offered for this decrease in coagulation time.

Hewson[2] in 1780 attributed the increased coagulability to a probable change in "that state of the blood vessels on which the thinness and lessened tendency of the lymph to coagulate depends; which surely is a very curious circumstance." Nasse[3] in 1842 and Brücke[4] in 1857 confirmed the observation as to the increased coagulability but showed at the same time a decrease in the fibrin content of the blood. This fact led them to conclude that the fibrin content and the rate of coagulation of the blood do not necessarily run parallel. Milian[5] in 1901 making his observations on hemorrhage following capillary puncture, thought that the increased coagulability was due to a local accumulation of a coagulating substance, stored in the skin and

[1] Gray, H. and Lunt, L. K.: American Journal of Physiology, 1914, xxxiv, 332.

[2] Hewson, W.: Experimental Inquiries into the Properties of the Blood, 1780, Part I, Experiment XX, 55.

[3] Nasse, H.: Handwörterbuch der Physiologie (Wagner), 1842, I, 75.

[4] Brücke, E.: Archiv für Pathologische Anatomie und Physiologie und für Klinische Medizin (Virchow), 1857, xii, 81, 172.

[5] Milian, G.: Mémoires de la Société de Biologie, 1901, liii, 556, 576.

in the tissues and released when they were wounded. Arloing[6] in the same year, experimenting with venous hemorrhage and finding the same shortening of coagulation time, opposed Milian's view of a local secretion of coagulating substance by the cells of the skin and tissues but suggested the possibility of an increase in fibrin ferment, resulting from an alteration in the blood when it came in contact with the lumina of the tubes which carried it from the veins to the receiving flasks. Arthus[7] in 1902 suggested that the acceleration of the coagulation time might be due, not to a quantitative increase in the amount of fibrin ferment in the blood after hemorrhage, but to an acceleration of its production. Hartman[8] in 1909 was unable to choose between diminished oxygen, augmented carbon dioxide, augmented fibrin ferment, and augmented flow of tissue thrombokinase. Von den Velden[9] in the same year explained the decreased coagulation time as the result of an augmented thrombokinase which reached the blood stream through an influx of tissue juice, and confirmed the observation of Nasse and Brücke that an immediate decrease in the fibrin content of the blood accompanies the decrease in coagulation time.

In order to throw further light on this interesting problem and to determine whether or not a quantitative alteration in the different factors of coagulation corresponding to a change in coagulation time might be observed, we undertook the series of experiments which are reported in this paper. In each experiment determinations of the coagulation times and analyses of the factors of coagulation were made before and after successive hemorrhages.

I. METHODS OF WORK

The technique of obtaining and examining the specimens of blood and of determining the coagulation times was as follows. The test animal was first anaesthetized with urethane (2 grams

[6] Arloing, F.: Mémoires de la Société de Biologie, 1901, liii, 675.

[7] Arthus, M.: Journal de Physiologie et de Pathologie Générale, 1902, iv, 273.

[8] Hartman, J.: Münchener Medizinische Wochenschrift, 1909, lvi, 796.

[9] Von den Velden, R.: Archiv für Experimentelle Pathologie und Pharmakologie, 1909, lxi, 37.

per kilo). The femoral or in some cases the carotid artery was next exposed, ligated distally and clamped proximally. A glass cannula washed with oxalate solution was then slipped into an oblique cut in the artery but not tied, the clamp was released, and the blood allowed to flow from the cannula. Blood obtained in this way supplied the specimens to be analyzed for the factors of coagulation and the specimens upon which coagulation time was determined. The specimens to be analyzed for the factors of coagulation were collected in glass graduates containing 1 per cent sodium oxalate in a 0.9 per cent sodium chloride solution in the proportion of 1 cc. of oxalate solution to 8 cc. of blood. A few drops of blood were first allowed to drop into a graduate containing the oxalate; the specimens upon which the coagulation time was determined were next collected in two test tubes (diameter 10 mm.), each graduated to hold 1 cc.; after which procedure the collection of the specimen to be analyzed was completed. The purpose of this order of work was to obtain for the determination of coagulation time a specimen of blood which had not stood in contact with the injured vessel wall and yet one which represented the condition of affairs in the body before the bulk of the hemorrhage occurred. The amount of the hemorrhage varied in different animals depending on their size. At the end of the bleeding the vessel was clamped proximally and the cannula removed.

As soon as the blood for determining coagulation time was collected, the tubes were placed in a water bath and kept at 37°–38°C. until they could be inverted without dislodging the clots. Observations were made by gently tilting the tubes at the end of five minutes and then at the end of every minute. Each coagulation time was taken with a stop-watch which was started as soon as the blood began flowing into the tube.

At an interval—usually of twenty minutes—the procedure described above was repeated, and a second determination of coagulation time (represented by the average of the two tubes) was made, and a corresponding oxalated specimen obtained to be analysed for the factors of coagulation. A fresh cannula or the old cannula freshly washed was used. This process of

bleeding at intervals of about twenty minutes was continued until the animal died. The later hemorrhages were of course smaller than the earlier ones, but varied in amount in different experiments.

After the death of the animal the specimens of oxalated blood were centrifugalized for 10 minutes at high-speed, and the plasma pipetted off. A portion of the plasma from each specimen was used to make *prothrombin* determinations; a portion was used to make *antithrombin* determinations; and a third portion (10 cc. if possible) was used to make *fibrinogen* determinations. In five experiments platelet counts[10] were made before each hemorrhage.

The methods of making prothrombin and antithrombin determinations were those described by Howell[11] and reviewed by Drinker and Hurwitz in an article not yet published. The fibrinogen determinations were made by the heat coagulation method.

II. EXPERIMENTAL DATA

Nine experiments were done on cats and eight on rabbits. In every experiment in which the coagulation time as a whole decreased there was a decrease in the amount of antithrombin. In all but two cases there was a steady fall in the fibrinogen content. The behavior of the prothrombin was irregular, on the whole tending first to increase slightly in amount and then to decrease. This irregularity in the prothrombin reaction depends to a certain extent upon the variations in antithrombin. With a fall in antithrombin one would expect a relative increase in prothrombin, though the absolute amount of the substance remained constant. In view of this fact the slight relative fall in prothrombin occurring in a number of our experiments in association with a fall in antithrombin, indicates a still greater absolute fall in the amount of prothrombin in the blood.

The following protocol illustrates the method by which our results were obtained. In this case the amount of prothrombin increased slightly while the amounts of antithrombin and fibrinogen decreased.

[10] Wright, J. H. and Kinnicutt, R.: Journal of the American Medical Association, 1911, lvi, 1457.

[11] Howell, W. H.: Archives of Internal Medicine, 1914, xiii, 76.

PROTOCOL—EXPERIMENT IV

Date, August 22, 1914.
Weight cat, 2 kilos. Urethane anaesthesia.
Anaesthesia begun, 11.40 a.m.
Operation begun, 12.03 p.m.

First Bleeding

Time, 12.11 p.m.
Amount, 23.5 cc.
Coagulation time (1) 14 minutes, 11 seconds.
(2) 13 minutes, 49 seconds.
Average, 14 minutes.

Second Bleeding

Time, 12.30 p.m.
Amount, 18 cc.
Coagulation time (1) 12 minutes, 12½ seconds.
(2) 14 minutes.
Average 13 minutes, 6¼ seconds.

Third Bleeding

Time, 12.50 p.m.
Amount, 18 cc.
Coagulation time (1) 12 minutes, 7 seconds.
(2) 12 minutes, 23 seconds.
Average 12 minutes, 15 seconds.
Death at 12.52 p.m.

ANTITHROMBIN DETERMINATION

Specimen 1

Thrombin drops	*Antithrombin drops*	*Time Interval minutes*	*Fibrinogen drops*	*Coagulation minutes*
3	1	15	10	15
4	1	15	10	10½
5	1	15	10	7¾
6	1	15	10	6
			Average,	9.81

Specimen 2

Thrombin drops	*Antithrombin drops*	*Time Interval minutes*	*Fibrinogen drops*	*Coagulation minutes*
3	1	15	10	13½
4	1	15	10	9½
5	1	15	10	6½
6	1	15	10	5¼
			Average,	8.62

Specimen 3

Thrombin drops	*Antithrombin drops*	*Time Interval minutes*	*Fibrinogen drops*	*Coagulation minutes*
3	1	15	10	6
4	1	15	10	5¼
5	1	15	10	4
6	1	15	10	3¼
			Average,	4.62

Control

Thrombin drops	*Fibrinogen drops*	*Coagulation minutes*
3	10	5
4	10	4

In making antithrombin determinations the selection of a definite end-point is somewhat difficult. The antithrombin delays or even prevents the formation of a solid clot. In our opinion the first definite appearance of coagulum in the clear solutions used is the safest end-point to employ, and we have used it throughout our determinations. In all of our experiments the average of the four antithrombin observations on each specimen has been used to represent the antithrombin factor in that specimen.

PROTHROMBIN DETERMINATION

Specimen 1

Oxalated Plasma drops	*0.5% $CaCl_2$ drops*	*Coagulation minutes*
5	2	5
5	3	5
5	4	4½
5	5	4½
5	6	4½

Tube containing optimum amount of $CaCl_2$ = 4½ minutes.

Specimen 2

5	2	3½
5	3	3½
5	4	3½
5	5	3
5	6	3

Tube containing optimum amount of $CaCl_2$ = 3 minutes.

Specimen 3

Oxalated Plasma *drops*	*0.5% $CaCl_2$* *drops*	*Coagulation* *minutes*
5	2	$3\frac{1}{2}$
5	3	$3\frac{1}{4}$
5	4	3
5	5	$2\frac{1}{2}$
5	6	$2\frac{1}{2}$

Tube containing optimum amount of $CaCl_2$ = $2\frac{1}{2}$ minutes.

The end-point in each prothrombin reaction is reached at the moment when the tube can be inverted without dislodging the clot. Great care must be taken not to jar the tubes while the clots are forming. In all of our experiments the prothrombin factor in each specimen is represented by the lowest figure in the series—i.e., the time required for clotting in the tube containing the optimum amount of calcium.

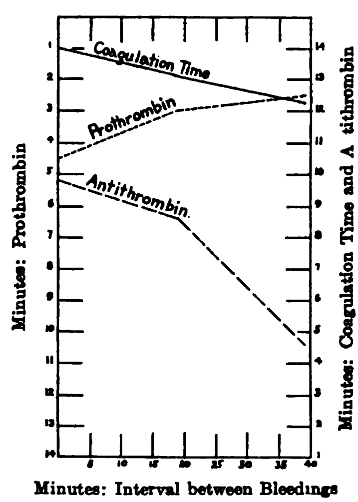

Fig. 1. Experiment IV.

FIBRINOGEN DETERMINATION

Specimen 1: 0.2137 gram in 100 cc. plasma.
Specimen 2: 0.1822 gram in 100 cc. plasma.
Specimen 3: 0.1350 gram in 100 cc. plasma.

Figure 1 (Exp. IV) represents the results of this experiment plotted graphically.

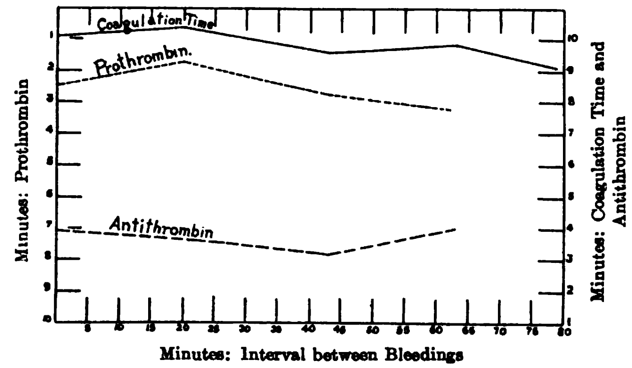

Fig. 2. Experiment IX.

Figure 2 (Exp. IX) represents the results of an experiment in which there was very little variation in the coagulation time, and a correspondingly small variation in the amount of antithrombin. The amount of fibrinogen increased slightly and then fell slightly, this experiment constituting one of the two exceptions to our observation of a steady fall in the amounts of fibrinogen. The amount of prothrombin rose slightly and then fell slightly, which in our experience was the most characteristic prothrombin behavior.

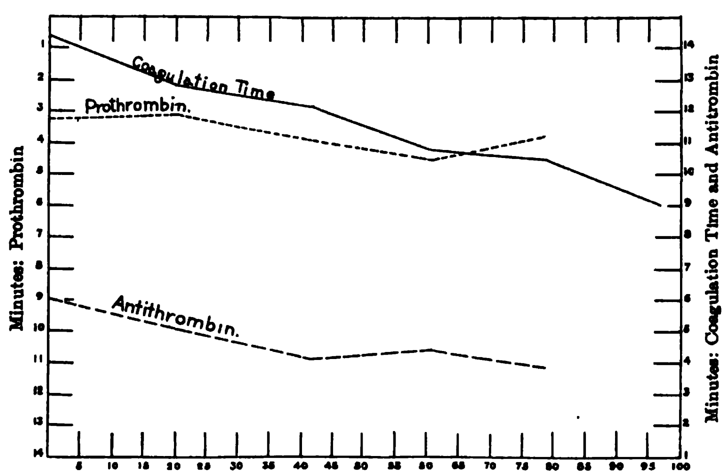

Fig. 3. Composite Curve, Cat.

Figure 3 is a composite curve embodying the coagulation times and the antithrombin and prothrombin determinations in our nine experiments on cats. The points to be noted are: (a) the fall in the amount of antithrombin corresponding to the decrease in coagulation time; and (b) a slight increase and then a slight fall in prothrombin. The final increase shown in the curve represents a single determination and is therefore unimportant. The fibrinogen determinations on cats are shown in Table 2, which contains a summary of the results of our entire series of experiments.

Discovering that at room temperature during a short period of contact with thrombin solution, the antithrombin in rabbits acts much more potently than does the antithrombin in cats, we determined to use the rabbit as a test animal.

The following protocol represents a typical rabbit experiment.

PROTOCOL—EXPERIMENT XV

Date, October 9, 1914.
Weight rabbit, 2.6 kilos. Urethane anaesthesia.
Anaesthesia begun, 10.35 a.m.

First Bleeding

Time, 11.22 a.m.
Amount, 13 cc.
Coagulation time (1) 18 minutes, 54 seconds.
(2) 18 minutes, 10 seconds.
Average 18 minutes, 32 seconds.

Second Bleeding

Time, 11.22½ a.m.
Amount, 13 cc.
Coagulation time (1) 18 minutes, 20 seconds.
(2) 18 minutes, 5 seconds.
Average 18 minutes, 12½ seconds.

Third Bleeding

Time, 11.47 a.m.
Amount, 12 cc.
Coagulation time (1) 15 minutes, 58 seconds.
(2) 15 minutes, 52 seconds.
Average 15 minutes, 55 seconds.

Fourth Bleeding

Time, 11.47½ a.m.
Amount, 12.5 cc.
Coagulation time (1) 14 minutes, 45 seconds.
(2) 16 minutes, 22 seconds.
Average 15 minutes, 33½ seconds.
Death at 11.55 a.m.

ANTITHROMBIN DETERMINATION

Specimen 1

Thrombin drops	*Antithrombin drops*	*Time Interval minutes*	*Fibrinogen drops*	*Coagulation minutes*
3	1	15	7	71¾
4	1	15	7	56
5	1	15	7	47¾
6	1	15	7	41¼
			Average,	54.18

Specimen 2

3	1	15	7	80½
4	1	15	7	62½
5	1	15	7	52
6	1	15	7	23½
			Average,	54.62

Specimen 3

3	1	15	7	57
4	1	15	7	46
5	1	15	7	33¾
6	1	15	7	15¼
			Average,	38

Specimen 4

3	1	15	7	64
4	1	15	7	47
5	1	15	7	33¼
6	1	15	7	23
			Average,	41.81

Control

Thrombin drops	*Fibrinogen drops*	*Coagulation minutes*
3	7	3½
4	7	2¼

PROTHROMBIN DETERMINATION

Specimen 1

Oxalated Plasma drops	*0.5% $CaCl_2$ drops*	*Coagulation minutes*
5	2	8¾
5	3	6½
5	4	8¾
5	5	7½
5	6	7¾

Tube containing optimum amount of $CaCl_2$ = 6½ minutes.

Specimen 2

5	2	8
5	3	6
5	4	7¾
5	5	7¼
5	6	9¼

Tube containing optimum amount of $CaCl_2$ = 6 minutes.

Specimen 3

5	2	11½
5	3	8¾
5	4	9¾
5	5	9¼
5	6	11¼

Tube containing optimum amount of $CaCl_2$ = 8¾ minutes.

Specimen 4

5	2	9¼
5	3	10½
5	4	10½
5	5	10
5	6	10½

Tube containing optimum amount of $CaCl_2$ = 9¼ minutes.

FIBRINOGEN DETERMINATIONS

Specimens 1 and 2 (mixed): 0.3240 gram in 100 cc. plasma.

Specimens 3 and 4 (mixed): 0.2475 gram in 100 cc. plasma.

Figure 4 (Exp. XVI) shows a marked decrease in coagulation time; a marked fall in antithrombin; a fall in fibrinogen; first a decrease and then a final increase in prothrombin.

Figure 5 (Exp. X) shows a decrease in prothrombin after two bleedings and then a slight increase. The fibrinogen has in-

creased slightly (0.0168 gram) instead of falling. The coagulation time and the antithrombin have both decreased steadily.

Figure 6 is a composite figure embodying the coagulation times and the antithrombin and prothrombin determinations in

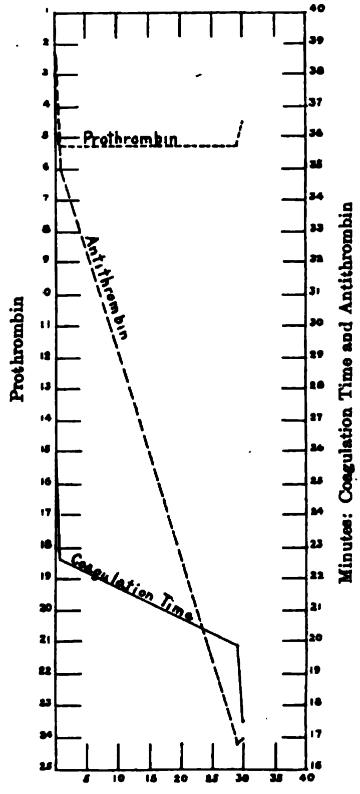

Fig. 4. Experiment XVI. Minutes 1 to 16 in the right hand column, being unnecessary in the figure, are omitted in order to save space.

our eight experiments on rabbits. It shows the same features which we have demonstrated in the composite curve of our experiments on cats: (a) a steady fall in antithrombin corresponding to a decrease in coagulation time; and (b) a slight increase, then a slight fall in the amount of prothrombin, and a final return to practically the original amount (two observations).

Our fibrinogen determinations on rabbits are shown in Table 2. The individual determinations vary less than those made upon cats but, with one exception, they show the same downward trend..

Thinking that possibly a marked change in the number of platelets after severe hemorrhage might be a factor to be con-

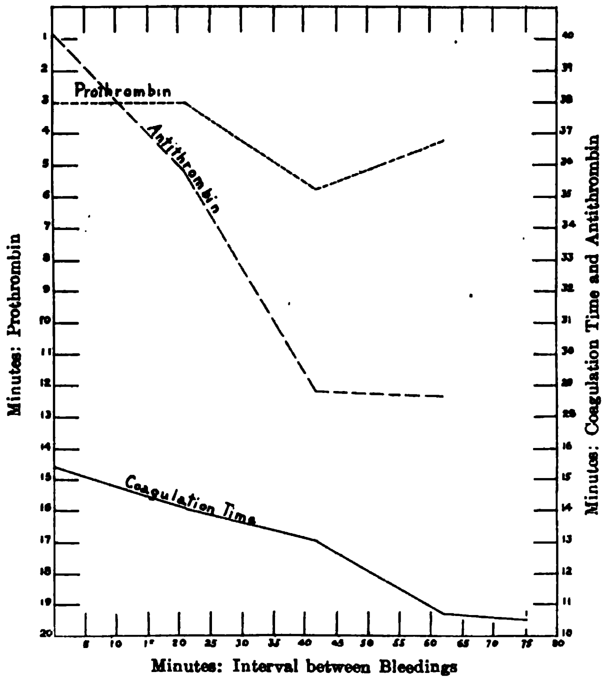

Fig. 5. Experiment X. Minutes 1 to 10 and 17 to 28 in the right hand column, being unnecessary in the figure, are omitted in order to save space.

sidered, we made platelet counts in five experiments before the first hemorrhage and then after each bleeding, but found practically no change in their number.

A comparison of the average results of our experiments on cats and those on rabbits is of interest. The behavior of the prothrombin in the two series of experiments was in general the same—a slight initial increase and then a slight decrease in

amount. In both series there was a steady fall in fibrinogen: in the cats an average decrease of 0.0762 gram per 100 cc. of plasma after three bleedings: in the rabbits an average decrease of 0.0770 gram after three bleedings and 0.0811 gram after four bleedings. The following table gives a comparison of the per-

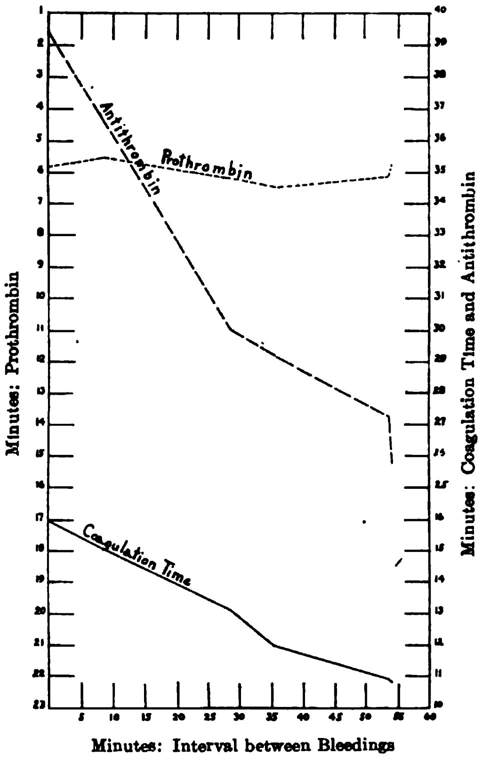

Fig. 6. Composite Curve, Rabbit. Minutes 1 to 10 and 17 to 25 in the right hand column, being unnecessary in the figure, are omitted in order to save space.

centages of the average decrease in coagulation times and in the amounts of antithrombin following each hemorrhage in the two series of experiments.

TABLE 1

	CAT		RABBIT	
	Percentage decrease in coagulation time	Percentage decrease in antithrombin	Percentage decrease in coagulation time	Percentage decrease in antithrombin
	per cent	*per cent*	*per cent*	*per cent*
2d specimen	11.1	15.1	5.4	6.7
3d specimen	15.4	30.9	17.8	23.6
4th specimen	25.0	26.4	24.8	25.9
5th specimen	27.0	34.6	31.6	30.8
6th specimen	37.4	No specimen obtained	32.2	34.7

Table 2 gives a summary of the results obtained in our entire series of experiments.

DISCUSSION AND CONCLUSIONS

Coagulation Time. Our experiments, in accord with the observations of other investigators, show that rapid progressive hemorrhage causes a decrease in coagulation time. An occasional animal proves an exception to this rule, and shows practically no change in its rate of clotting no matter how severe the hemorrhage (e.g., Exps. II and XI in Table 2), but the majority of the animals show a gradual steady fall in the rate of coagulation as the hemorrhage proceeds.

Antithrombin. In our series of experiments, the decrease in coagulation time is accompanied by a decrease in the amount of antithrombin. Whether or not this change is the result of a simple dilution of the blood by an influx of fluid from the tissues, or whether it is due to a decrease in the amount of antithrombin formed, we are unable to say. In either case the fact of diminution remains. Gray and Lunt[12] have shown that there is no decrease in coagulation time after hemorrhage in an anterior animal (i.e. an animal in which the aorta and vena cava are ligated just above the diaphragm). This observation may be easily reconciled with our experiments. If the decrease in antithrombin

[12] Gray and Lunt: Loc. Cit. (1).

TABLE 2

Cat

Specimens	COAGULATION TIME 1	2	3	4	5	6	INTERVAL BETWEEN BLEEDINGS 1–2	2–3	3–4	4–5	5–6	ANTITHROMBIN 1	2	3	4	5	6	PROTHROMBIN 1	2	3	4	5	6	FIBRINOGEN 1	2	3	4
	min. sec.	*min. sec.*	*min. sec.*	*min. sec.*	*min. sec.*	*min. sec.*	*min.*	*min.*	*min.*	*min.*	*min.*	*min.*	*min.*	*min.*	*min.*	*min.*	*min.*	*min.*	*min.*	*min.*	*min.*	*min.*	*min.*	*gm.*	*gm.*	*gm.*	*gm.*
Exp. I	11 38	10 36½	9 45	7 44			14	30	19			7.56	6.00	5.50	5.00			2	2	4	4½			Dete	rmina	tion	Lost
Exp. II	10 35¾	10 11	10 0	9 ¾			16	15	15			Deter	minat	ion L	ost			2½	2½	2	4½			0.4758	0.4016	0.3408	
Exp. III ...	19 0	12 27	14 54½	15 7			28	16	19			6.75	6.50	4.87	4.87			3½	3	3¼	5			0.0675	0.0652	0.0644	
Exp. IV. ..	14 0	13 6¼	12 15				19	20				9.81	8.62	4.62				4½	3	2½				0.2137	0.1822	0.1350	
Exp. V.....	15 27½	11 16	11 31½	11 35			20	20	20			Deter	minat	ion L	ost			2½	4¼	3½	5			0.3611	0.3116	0.2430	
Exp. VI....	15 16½	12 59½	10 47				20	29				4.50	3.68	3.62				5	3½	3½				0.2216	0.1049	0.0974	
Exp. VII. .	19 34½	20 44½	18 12½				23	23				4.12	3.31	3.18				3	5	8¾				0.5342	0.5062	0.4134	
Exp. VIII..	14 0	13 33	12 32	11 35	11 54½	9 1	20	21	20	20	18	5.43	4.00	4.12	3.81	3.93		4	3½	4½	5	3¾		0.0843	0.0843	0.0562	0.0481
Exp. IX....	10 4	10 20	9 35½	9 49½	9 6		20	23	20	16		3.93	3.62	3.18	4.00			2½	1¾	2¾	3¼			0.2373	0.2666	0.2361	
Average in minutes	14.4	12.8	12.17	10.8	10.5	9.01	20	21.88	18.83	18	18	6.01	5.10	4.15	4.42	3.93		3.27	3.13	3.86	4.54	3.75		0.2744	0.2403	0.1982	0.0481

Rabbit

Specimens	COAGULATION TIME 1	2	3	4	5	6	INTERVAL BETWEEN BLEEDINGS 1–2	2–3	3–4	4–5	5–6	ANTITHROMBIN 1	2	3	4	5	6	PROTHROMBIN 1	2	3	4	5	6	FIBRINOGEN 1	2	3	4
Exp. X.....	15 26	14 4½	13 2½	10 45½	10 31		21	21	20	13		40.18	35.81	28.80	28.68			3	3	5¾	4¼			0.2925	0.2947	0.3093	
Exp. XI....	15 5½	14 35	15 17				20	21				23.06	24.50	20.93				2¼	2½	3¾				0.3240	0.2812	0.2328	
Exp. XII...	15 56	13 34	9 3	12 41	12 28	12 25	½	21½	½	21	½	41.50	39.68	32.43	33.56	30.12	25.56	5¼	6½	8	6	6½	6	Deter	minat	ion n	ot made
Exp. XIII..	12 16	11 54	8 8½	8 55	9 48	9 15	½	20½	½	20½	½	36.06	36.37	28.68	23.81	24.43	25.87	8½	6	5¾	6¾	5¾	5½	Deter	minat	ion n	ot made
Exp. XIV..	9 37	11 17	9 39	9 21			21	½	24			38.12	31.43	39.06	23.12			9½	8¾	4½	4¾			Deter	minat	ion n	ot made
Exp. XV...	18 32	18 12½	15 55	15 33½			½	24½	½			54.18	54.62	38.00	41.81			6½	6	8¾	9¼			0.3240	0.3240	0.2475	0.2475
Exp. XVI..	25 55	22 38	19 53	17 30½			½	28½	1			38.62	35.06	16.75	16.87			4¾	5¼	5½	4½			0.2621	0.2621	0.1968	0.1968
Exp. XVII.	15 4	14 40	14 4	9 15			2	24½	1½			43.75	36.56	36.18	36.68			6¼	6½	7¾	9¼			0.5062	0.5062	0.3375	0.3375
Average in minutes	15.98	15.11	13.12	12.00	10.92	10.83	8.25	20.25	6.85	18.16	0.5	39.43	36.75	30.10	29.21	27.27	25.71	5.81	5.56	6.18	6.46	6.12	5.75	0.3417	0.3336	0.2647	0.2606

which we have observed is due to a decrease in the amount formed, we may assume that antithrombin is formed in one of the abdominal organs. In support of such an assumption there is considerable evidence to show that the liver is very active in antithrombin production.

If the decrease in antithrombin is a matter of dilution, we know that the major portion of the fluid entering the blood after hemorrhage comes from the abdominal region. This fluid which enters the blood after hemorrhage is, in our opinion, lymph, not tissue juice. If it could be shown that tissue juice—the fluid obtained from wounded tissues—entered the blood after hemorrhage, we could readily explain our diminished amount of antithrombin by assuming that a large portion of it was neutralized or fixed by the thromboplastin in the tissue juice. In this way the amount of free antithrombin in the blood would be diminished, and our tests for it would show this reduction. But lymph, on the contrary, is not rich in thromboplastin. Howell[13] has shown very recently that lymph contains prothrombin and antithrombin in the same concentration as blood plasma, but much less thromboplastin. There is, therefore, a relative excess of antithrombin in lymph which explains its long coagulation time. Addition of thromboplastic material (tissue extract or kephalin solutions) causes lymph to clot promptly and firmly. In view of these facts it would seem that an influx of lymph into the blood after hemorrhage would bring with it a relative excess of antithrombin, which would increase the coagulation time of the blood instead of decreasing it. It is perhaps more reasonable to suppose that the decrease in antithrombin which we have demonstrated is the result of diminished antithrombin production.

Prothrombin. The behavior of the prothrombin in our experiments varied in different animals: occasionally it increased steadily in amount, occasionally it decreased steadily, but in the majority of cases it first increased slightly and then decreased slightly. The only conclusion in regard to the prothrom-

[13] Howell, W. H.: American Journal of Physiology, 1914, xxxv, 483.

bin which we feel warranted in making is that the prothrombin changes in our series of experiments do not offer any explanation for the decrease in coagulation time which occurred.

Fibrinogen. The fibrinogen in our experiments, as estimated by the heat coagulation method, gave marked variations as to the amount present in different animals, a fact in accord with Whipple's observations,[14] but remarkably uniform results as to the effect of hemorrhage on the fibrinogen content of the blood of individual animals.

As time has passed we have felt more and more certain that this method of estimating fibrinogen is open to question. In Table 2, it may be noted that while one cat shows a normal content of 0.0675 gram of fibrinogen per 100 cc. of plasma, another cat shows a normal content of 0.5342 gram. These figures give the maximal variation, 0.4667 gram, which we found between two different animals. Whipple's maximal variation in dogs was 0.6686 gram per 100 cc. of plasma, but he never obtained in normal animals the very low figures of 0.0675 gram and 0.0843 gram which we encountered twice in this series of experiments, and which we have found a number of times in some experiments on rabbits not yet reported. None of these animals possessing an apparently very low fibrinogen content showed any bleeding tendencies but seemed normal and healthy in every way. We have taken every precaution to assure ourselves that there has been no technical error in the application of the method, and we are convinced that there is enough variation in the reactivity of fibrinogen to heat to render the heat coagulation method of determining this substance of somewhat questionable accuracy.

Our data in regard to fibrinogen is as follows. With the exception of two cases (Exps. IX and X) all of our determinations showed a steady decrease in the amount of fibrinogen following each hemorrhage. The average fall from the normal in all cases of specimens obtained at the third bleeding was 0.0766 gram per 100 cc. plasma.

[14] Whipple, G. H.: American Journal of Physiology, 1914, xxxiii, 50.

Dreyer[15] has shown that the fibrinogen content of the blood increases after hemorrhage provided the interval between bleedings is a period of twenty-four hours. It is possible that in our experiments the apparent reducing effect of rapid progressive hemorrhage upon the fibrinogen content of the blood, is in reality a false one. It may be that instead of an actual fall in the amount of the fibrinogen, there occurs some alteration in the fibrinogen itself which diminishes its responsiveness to the heat coagulation test. On the other hand we must remember that Nasse, Brücke, and von den Velden have all reported an immediate diminution in the fibrin content of the blood following severe hemorrhage, and at the same time an increase in the speed of coagulation.

There remains to be considered the question as to whether or not the results which we have obtained in our analyses of the factors of coagulation after hemorrhage are sufficient to explain the decrease in coagulation time which occurred, but this question unfortunately must remain unanswered. A decrease in antithrombin will of course favor more rapid clotting, but whether or not the decrease which we have shown is sufficient to compensate for a certain amount of diminution in prothrombin and for a possible diminution in fibrinogen also, we are unable to say. The fact that there was very little change in antithrombin in two of our experiments in which the coagulation time was practically constant seems suggestive in this connection.

We believe that the chief interest in these experiments lies in our demonstrations of a variation in antithrombin. Except for the experiments of Davis[16] showing the production of an antithrombin wave as the result of thrombin injections and the work of various investigators on the increase in antithrombin as the result of peptone injections, all previous work on the antithrombin in the blood has indicated that this substance is remarkably constant. Thrombin and peptone injections produce

[15] Dreyer, G. P.: Studies from the Biological Laboratory of the Johns Hopkins University, 1893, v, 77.

[16] Davis, D.: American Journal of Physiology, 1911, xxix, 160.

an increase in the amount of antithrombin. Our experiments are the first instance in which there has been reported a positive decrease in the antithrombin content of the blood.

SUMMARY

1. Rapid progressive hemorrhage causes a progressive decrease in coagulation time. An occasional animal proves an exception to this rule and shows no change in its rate of clotting, no matter how severe the hemorrhage.

2. Antithrombin decreases in amount when the coagulation time decreases and remains practically constant when the coagulation time is unchanged.

3. Prothrombin tends first to increase slightly in amount and then to decrease slightly.

4. Fibrinogen, estimated by the heat coagulation method, decreases as bemorrhage progresses.

5. Platelets counts do not vary with rapid progressive hemorrhage.

We wish to thank Dr. W. B. Cannon for his interest and advice.

THE VASOMOTOR NERVES OF THE PORTAL VEIN

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The experiments of Mall[1] pertaining to the influence of the portal system upon the distribution of the blood, have been cited repeatedly in proof of the contention that the portal vein is controlled by vasomotor nerves. The observation which is generally regarded as adequate to fully substantiate this conclusion, consists in the fact that high occlusion of the abdominal aorta fails to destroy the rise in the carotid blood pressure which usually accompanies splanchnic stimulation. Mall states that "Wenn sich die Aorta so verstopfen lässt, dass den Arterienästen unterhalb des Zwerchfells kein Blut mehr zufliesst, und wenn dann die Reizung des Nervus splanchnicus den Druck in der Carotis noch steigert, so ist damit streng bewiessen, dass nur die aus dem Portalsystem durch das Herz in das Aortenwerk eingeführten Blutmassen den Druck erhöht haben."

It is the purpose of the present discussion to show that this "proof" is open to several serious objections. While, no doubt, an augmentation in the carotid blood pressure can be obtained under the conditions here briefly outlined, the conclusion based upon it, that the portal vein is innervated by veno-motor nerves, must be regarded as not having been substantiated.

Mall states that no blood can reach the arteries below the diaphragm, if the aorta is occluded by a ligature at a point directly below the origin of the arteria subclavia sinistra. This ligation having been effected, the crural artery will always be found free from blood, but such a result cannot be attained if the aorta is obstructed immediately above the diaphragm.

[1] Archiv für Anat. und Physiologie, 1892, 409.

The statement is also made that the occlusion of this blood vessel is compensated for in the course of time. Quite similarly, stimulation of the splanchnic nerves results in an augmentation of the general blood pressure, but the rise is more marked if the posterior part of the body is first rendered "bloodless." Thus, values of 200 mm. Hg. are seldom reached with the aorta intact, while the compression of this blood vessel enables the pressure to rise to points considerably above this level. In accordance with Mall this secondary rise finds its origin in a rapid influx of blood from the portal vein occasioned by the constriction of this blood vessel.

Contrary to Mall it has been demonstrated by Vehlich[2] that a certain amount of blood will find its way into the crural artery of the dog even after the aorta has been ligated below the subclavian artery. A similar result has been obtained by Hering[3] who states that this procedure renders the arteries of the abdominal cavity of rabbits only partially bloodless, i.e., "beim Aufschneiden fliessen immer noch ein bis zwei Tropfen Blut ab."

The conclusion to be drawn from these experiments is that the compression of the aorta at the point indicated does not lead to a confinement of the blood in anterior channels, but rather favors its escape from them by opening up a very effective system of anastomosing blood vessels. By dividing the body transversely Vehlich has shown that these connecting blood vessels are located within the vertebral column. It must be admitted, however, that the establishment of this fact by physiological means has not augmented our knowledge materially, because this anastomosis seems to have been clearly recognized many years before the time of publication of the article of Vehlich and even before the appearance of the paper of Mall. Thus, Ellenberger and Baum[4] state that the arteria spinalis anterior passes to the posterior end of the cord by making use of the sulcus longitudinalis anterior as its highway. This blood vessel receives connecting branches from the intercostal, lumbar and sacral

[2] Pflüger's Archiv, xcv, 1903, 264.
[3] Pflüger's Archiv, lxxii, 1898.
[4] Anatomie des Hundes, Berlin, 1891.

arteries. Moreover, Heidenhain[5] made the observation that occlusion of the aorta does not always provoke a fall in the crural blood pressure to zero and concluded, therefore, that an arterial communication is present somewhere in the domain of the spinal column.[6]

The existence of an anastomosis of this kind is also made very probable by the experiments of Basch and Oser.[7] Having ligated the aorta above the diaphragm, these authors injected nicotine into the current of the carotid artery. Very pronounced contractions of the intestine resulted in consequence of this procedure which, however, were interpreted as proving that this drug possesses a central action. Under quite similar experimental conditions Pal[8] employed morphine to test the effect of this agent upon the centers of the splanchnic nerves. Luchsinger[9] believed that the action of pilocarpin in causing a secretion of sweat upon the paws of the cat's foot, is entirely central, because this effect could also be obtained after ligation of the abdominal aorta.

This deduction, however, does not seem to be justified, because Robillard[10] has shown that the injection of pilocarpin does not produce a secretion of sweat if the tibial nerve is made to form the only connection between the foot and the remaining portion of the posterior extremity. A true central stimulation, as may be had, for example, by an accumulation of carbon dioxide, will, however, readily produce a secretion of sweat at this time. Sodium iodide injected into the carotid artery or jugular vein of dogs, could easily be shown to be present in the blood of the crural artery after ligation of the aorta. It could also be detected after these animals had been divided transversely in such a way that the spinal column formed the only connecting bridge between the anterior and posterior segments of their bodies. This proof of Vehlich has been greatly amplified by him. For

[5] Pflüger's Archiv, xlix, 226.
[6] Compare: Mares, Pflüger's Archiv, xlvii, 1903, 567.
[7] Wiener: Med. Jahrbücher, 1872.
[8] Wiener: Med. Presse, 1900, No. 45.
[9] Pflüger's Archiv, xv, 1877, 482.
[10] See Biedel, Wiener Med. Presse, 1899.

example, he has been able to incite rises in the crural blood pressure by injecting adrenalin into the vascular circuits of the fore part of the body and has succeeded in recovering from this blood vessel coloring material that had been added to the carotid blood stream.

These experiments, as well as others that might still be mentioned, prove conclusively that the ligation of the thoracic aorta, as practised by Pal and Mall, does not lead to a cessation of the circulation through the channels located below the diaphragm. Hence, all conclusions which are based upon an assumption contrary to this fact, cannot be regarded as being in agreement with well established anatomical and physiological data.

The second discrepancy pertains to the size and location of the vascular field which is controlled by the splanchnic nerves. The conclusion of Mall relative to the motor power of the portal vein, is based upon the phenomenon that stimulation of the greater splanchnic nerves induces rises in the carotid blood pressure even after the thoracic aorta has been ligated. The claim is made that the constriction of this bloodvessel causes a transfer of portal blood into the arterial channels sufficient to produce this change. It must be evident that if the augmentation in the carotid pressure is to be regarded as an indication of an active reduction in the size of the portal bloodbed, the vascular field governed by the splanchnic nerves, must be carefully restricted to this venous channel. The question whether Mall has actually succeeded in narrowing the area experimented upon in a satisfactory manner must be answered negatively.

Based largely upon the presentation of Swan,[11] it is stated by Mall that each splanchnic nerve arises by three separate strings, namely, as the splanchnicus major, minor and tertius. Dense networks of fibres appear in the vicinity of the adrenal bodies, from which others continue onward to aid in the formation of the superior mesenteric and coeliac masses of the solar plexus. To quote further: "Ausser den Zweigen zum Darm kanal und seinen

[11] Comparative anatomy of the nervous system, London, 1864.

Drüsen sendet das Ganglion noch zuweilen solche zur Niere." Obviously, the picture which is drawn here regarding the distribution of the splanchnicus is very indefinite, and is much less accurate than the description given upon page 569 of Ellenberger and Baum's *Anatomie des Hundes*. As this work has been cited by Mall, its contents pertaining to the sympathetic innervation of the abdominal parts, must or should have come to his attention.

While still incomplete, the description of Ellenberger and Baum is not at all inconclusive or evasive regarding the sympathetic innervation of the kidneys. Contrary to the preceding quotation, it is stated here very clearly that the "rami renales kommen aus der caudalen Seite des Geflechtes—und bilden im Verein mit Zweigen vom Ganglion mesentericum superius um die Nierenarterie den Plexus renalis; die Fäden desselben umspinnen die Nierenarterien und dringen in den Nierenhilus ein." These authors even mention the fact that the splanchnic nerves supply the supra-renal bodies; and possess other intra-abdominal ramifications.

It must be conceded, therefore, that the nerves in question control a vascular field which is very much larger than that drained by the portal vein. Hence, when the stimulation of the splanchnic nerves incites an augmentation in the carotid blood pressure, this rise cannot be said to have been caused solely by constrictive reactions in the portal vein. In accordance with the evidence just submitted, they may indeed be referred to motor activities in several other circuits possessing a sympathetic nerve supply. The possible contention that, if compared with the portal blood flow, these extra-portal blood streams become of negligible importance, cannot justly be offered in support of the conclusions of Mall, because a brief comparison of the blood supply of the kidneys with the portal bloodflow will show the opposite to be true. In accordance with the calibrations furnished by Burton-Opitz,[12] a liver weighing 500 grams receives about 7.0 cc. of blood in a second or 420 cc. in a minute. Of

[12] Quarterly Journal of Experimental Physiology, v, 1912, 189.

this total amount nearly three-fourths are gathered from the portal organs. A liver of this weight we may expect to find in a dog weighing about 15 kg. and, as the total quantity of blood present in an animal may be roughly calculated at one-thirteenth of the body weight, the entire mass of blood must traverse this organ once in every three minutes.

When reference is had solely to the total blood supply of organs, the liver no doubt ranks first among the structures of the body, but if especial emphasis is placed upon the amount of blood that is allotted to each 100 grams of substance in a unit of time, this organ cannot be said to be as vascular as the kidney. Upon this basis, the combined arterial and venous supply of the liver amounts to only 1.40 cc. in a second or to 84 cc. in a minute, while the venous stream alone possesses a value of 59 cc. in a minute. Opposed to this figure, we have a minute-volume for the renal vein of 151 cc. per 100 gram of kidney-substance.[13] Clearly, therefore, the kidney is an organ that must be reckoned with. It is compact and, while it does not contain a considerable quantity of "residual" blood, it is capable of taking up large amounts of blood by simply permitting the latter to flow through its channels without much hindrance. Thus, we know that the renal vein frequently discharges a quality of blood which is markedly lighter than that contained in the vena cava; its color, in fact, often closely approaches that of the arterial blood. Excepting the thyroid body, the kidney is the most vascular structure in the body and naturally, the value given previously gains much in importance, if it is doubled to represent the amount of blood discharged by the two organs.[14]

It has been shown by Burton-Opitz and Lucas that the shutting off of the arterial supply from one of these organs suffices to cause an augmentation in the general blood pressure. Similarly, it has been proven by them that this result may also be attained by excitation of the greater splanchnic nerves. Hence, the quan-

[13] See: Burton-Opitz and Lucas, Pflüger's Archiv, cxxiii, 1908, 156.

[14] In a dog weighing about 16 kg. each kidney discharges 1.64 cc. of blood in a second. The weight of each organ amounts to 65 grams; hence, each 100 grams of substance receive 151 cc. of blood in a minute.

tity of "residual" blood which is discharged under these conditions by the constricting renal bloodvessels, must indeed be considerable. If now it is taken into account that Mall has also disregarded the haemodynamical influence of other extra-portal structures; for example, that of the supra-renal bodies, it becomes quite evident that the rises in the carotid blood pressure obtained under the conditions previously outlined, cannot be referred solely to constrictive reactions in the portal vein.

In order to amplify his results Mall has also resorted to excitation of the splanchnic nerves after temporary occlusion of the portal vein and vena cava below the diaphragm. The carotid pressure was observed to decline subsequent to the ligation, but was seen to rise during the period of stimulation. In accordance with this result the statement is made that the splanchnic nerves undoubtedly possess the power of removing the blood from the liver. The stimulations were confined at first to the different roots of the splanchnic nerve, but a record was also made of the excitation of the peripheral end of the network of fibres accompanying the hepatic artery.

Whether the conclusion just quoted has been satisfactorily proven by the tests just briefly outlined, must remain doubtful, because no data are given regarding the exact location of the ligatures. The anastomosis between the different portal tributaries, as well as between this system and more central channels, is very complete. We have seen previously that one of the essential preliminary procedures, namely, the obstruction of the thoracic aorta, has failed in its purpose on account of the presence of anastomosing arterial channels. It has been proven that a cessation of the arterial influx cannot be accomplished in this way.

Concurrently, no reason seems to be at hand to assume that the object which Mall hoped to attain by obstructing the inferior cava, has actually been attained. In fact, it is admitted in the paragraphs now under discussion that the vena portae is connected with the heart by more than one channel. It is stated here that the path of least resistance leads through the liver, and the one of greatest resistance through the vena azygos. Mall also

shows that a communication exists between the mesenteric veins and the vena azygos.

Resort was also taken to the ligation of the inferior cava above the diaphragm and hence, presumably, centrally to the orifice of the hepatic vein. When this blood-vessel was obstructed in addition to the thoracic aorta, the excitation of the splanchnic nerves failed to induce the characteristic augmenting effect in the carotid blood pressure. In fact, this procedure was invariably followed by a decline in the general blood pressure. In illustration of this result I quote the figures of a part of experiment II. To begin with, the carotid pressure amounted to 134 mm. Hg. Measured at intervals of 10 seconds, the ligature of the cava above the diaphragm induced the following values: 104, 80, 47, 36, 22, and 14 mm. Hg.

While the absence of an augmentation in the carotid blood pressure fails in this case to strengthen the assumption that the portal vein possesses constrictive powers, it proves very suggestive in another way. To be sure, any confinement of the blood in anterior vascular channels produces at first a decided rise in the carotid blood pressure which, however, is compensated for in a measure within a reasonable time thereafter. It appears to me that the fall in blood pressure of 120 mm. Hg obtained within a period of 60 seconds, does not point so much towards a compensatory approximation of the anterior vascular channels as towards a rather free leakage of blood into the supposedly tight vascular compartments posterior to the ligatures. This result might indeed, have suggested to Mall that the occlusion of the thoracic aorta does not prevent the blood from escaping into posterior bloodvessels.

In referring to the description of the splanchnic nerves by Ellenberger and Baum, the statement is made by Mall that: "In letzterem Werke werden abweichend von meinen Befunden statt eines drei Plexus supra-renales beschrieben." I quote from page 569 of this work as follows: "Nahe dem dorsalen Rande derselben (Nebenniere) nimmt der N. splanchnicus ein Ganglion in seine Bahn auf, von dem ein stärkerer oder zwei schwächere Zweige nach dem Ganglion coeliacum, das zur Seite

der A. coeliaca liegt, abgehen, während ein anderer Zweig sich mehr caudal wendet und noch ein bis zwei kleinere Ganglien aufnimmt. Dabei verbindet sich dieser Zweig mit den drei splanchnici minores und bildet mit ihnen gemeinsam das an der medialen Fläche der Nebenniere gelegene Nebennierengeflecht. (Plexus supra-renalis). Aus demselben und zwar wesentlich aus seinen Ganglien gehen, etc." I venture to believe that a scrutiny of this description will show that Ellenberger and Baum should not be charged with having recognized three plexus suprarenales. The fact that they have not fallen into so obvious an error is shown especially well towards the end of this description, where they consistently refer to this plexus by the singular term.

I would also call attention to another misconception. If it is granted that stimulation of the peripheral end of the hepatic plexus leads to the removal of a certain quantity of blood from the liver even after the vena portae has been ligated, the deduction might seem justifiable that this bloodvessel possesses motor activity. Such a conclusion, I venture to believe, is not warranted upon the basis of Mall's experiments, because the liver derives its blood from two sources. Obviously, therefore, the preceding deduction can be accepted as proven only under the condition that the arterial supply has been prevented from reaching this organ. In the first place, considerable histological evidence is at hand to show that the arterial and portal terminals communicate with one another in the liver.[15] Secondly, it has been established by Burton-Opitz[16] that the occlusion of the portal channel at the hilus does not lessen the blood supply of this organ in a corresponding measure, but whenever such a procedure is attempted, an increase in the arterial influx results which in part compensates for the quantity of portal blood lost.

My objections to the conclusions now under discussion are only technical, however, because I myself have shown that the intra-hepatic bloodvessels are equipped with motor mechanisms. One of these is situated in the terminals of the hepatic artery

[15] See Gad, Dissertation, Berlin, 1873. Also Köllicker's Gewebelehre.
[16] Quarterly Journal of Experimental Physiology, ii, 1911, 93.

and the other in the radicles of the portal vein.[17] This result has been arrived at (a) for the arterial channels under exclusion of the portal blood, and (b) for the venous tubules under exclusion of the arterial influx. Again, I have sought to establish the existence of these mechanisms in each case separately by calibration of the arterial and portal blood streams before and during the stimulation of the hepatic nerve fibres, as well as before and after the administration of varying quantities of adrenalin.

In this connection mention must also be made of the fact that the term "portal vein," as formerly used, no longer conveys a clear impression regarding the exact seat of the vasomotor reactions. Reference may be had, on the one hand, to the truncal portion of the vein and, on the other, either to its terminals in the liver or to its radicles in the different organs forming the portal system. While we know that the arterial entrance to the portal vein is well guarded peripherally by very efficient motor mechanisms located within the domain of the different "portal" organs, it cannot be said that the existence of such a mechanism in the truncal segment of this channel has been definitely established. I believe that in the absence of more specific statements, Mall's references to the portal vein are usually thought to apply to the trunk of this bloodvessel.

I venture to believe that the statements of Mall quoted in this paper, have been disproven in a satisfactory manner without that a further discussion of minor errors becomes necessary. As has been emphasized previously, my principal objections against the conclusions of Mall are (a) the improper limitation of the vascular fields experimented upon (b) the only partial degree of anaemia which ligation of the thoracic aorta produces, and (c) the imperfect localization of the reactions for which the intra and extrahepatic anastomosis of the blood-vessels are responsible.

[17] Quarterly Journal of Experimental Physiology, vii, 1913, 57.

THE INFLUENCE OF PUPILLARY DIAMETER ON VISUAL ACUITY

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INTRODUCTORY

One of the commonly accepted functions of the pupil of the eye is that of compensating its dioptric defects. Owing to the chromatic and spherical aberration of the refractive system of the eye the light from a bright point of an outer object does not come accurately to a focus upon the retina, but forms there a small diffusion circle of light, which if large enough has blurred vision as its effect. Astigmatism adds to this effect, and if irregular introduces additional irregularities, causing still further imperfectness of the image. The pupil by reducing the size of the light-pencil, reduces also the size of the diffusion-circle and other irregularities, gives a clearer image and consequently better vision. The present work was undertaken to estimate the importance of this function of the pupil and further to find by experiment how far the eye is actually subject to that which physicists designate as the resolving power of its optical system. The image of a bright point, such as a star, when formed by the object-glass of a telescope, and looked at through a highly magnifying eye-piece, is seen not to be a mere point of light, but a central point of light in a concentric series of dark and bright annuli, each bright ring being of greater intensity than those surrounding it, and the central point being the brightest part of the figure. This pattern is due to diffraction and interference of the light waves passing through the lens-aperture, and is not to be confounded with the diffusion circle previously spoken of, for it is something wholly different. A detailed explanation of its origin can be found in the text-books on physical optics (1), (2), (3).

It is sufficient to state here that it has been empirically found that the eye is just able to differentiate two such images when the central point of one falls in the darkest part of the inmost dark ring of the other, independently of the magnification introduced by the eye-piece. The condition of this is expressed by the formula.

$$\theta = 1.22 \frac{\lambda}{D}$$

Where θ = the angular separation of the two bright points subtended at the optical center of the image-forming system $\left(\text{expressed as } \frac{\text{arc}}{\text{radius}}\right)$,

λ = the wave-length of the light concerned,

and D = the diameter of the lens-aperture (these last two being expressed in equal units).

Inspection of the formula shows that the larger D is, the smaller is θ. That is, the resolving power is proportionately greater, the larger the aperture. This is contrary in sense to the usually accepted view as to the effect of the pupil of the eye, and presupposes a lens system free from defects. From this it would at once appear possible, by using a sufficiently small diaphragm before the eye, to so reduce the effectiveness of its optical imperfections, and at the same time decrease its resolving power as explained above, that the eye would be subject chiefly to the latter, and that under certain conditions at least a smaller aperture would actually give lower visual acuity than a larger one. The present work shows that this effect can be obtained, and just how far it is effective.

HISTORICAL

The writer has found in the literature only two systematic investigations on the effect of pupillary width upon visual acuity. Uhthoff (4) in 1890 studied this question as a preliminary one to determine the optimal size of an artificial pupil to use in the investigation of visual acuity in spectral light of different wavelengths and intensities. He used diaphragms of various sizes

(1.06 to 3.02 mm. diameter) and altered the slit-width of his spectrometer system inversely as the area of the diaphragm used, so as to get equal luminous flux into the eye in all cases. He found that under high illumination a certain diaphragm of 2.06 mm. diameter gave the best vision, the next larger (3.02 mm.) and next smaller (1.55 mm.) giving lower values. In other sets of observations at lower brightness (less energy admitted to the eye) smaller apertures gave optimal results. Vision of course was lower throughout these sets. The results were given for wave-lengths 505 $\mu\mu$ and 605 $\mu\mu$, in both cases showing the facts just stated. He attributed this effect to diffraction, noting that with a 1 mm. aperture the limbs of the test-object appeared indistinct and broader as the limit of vision was reached, whereas with the larger diaphragms they continued to be sharply outlined up to the point of unrecognizability. He concluded that the diaphragm of 2.06 mm. diameter was the best one to use, as above that the optical irregularities of the eyes were in evidence.

Hummelsheim (5) in 1898 used homatropin and pilocarpin to vary the size of the pupil, and determined visual acuity by means of the Snellen illiterate character E under daylight illuminations varying from 1 to 200 mk.[1] The illumination was controlled by window shades and measured with a Weber photometer. His results showed visual acuity with the contracted pupil to be higher and with the dilated pupil lower than with the normal pupil, the differences showing a tendency to disappear at low illuminations, under which conditions vision dropped to lower values in all cases. The smallest pupil worked with in his two observers was 1.5 mm. in the case of both eyes of the one and 2.25 mm. in the other.

[1] Meter-Kerzen. The candle referred to is the Hefner unit; approximately 0.9 international candle.

THE PRESENT WORK

Apparatus

The test object used is one which has already been described (6) [see also (7) (8)] and permits of continuous variation in the width of a set of parallel bands without altering any other factor in the stimulus. The average brightness of the test field is equal for all widths of the bands. The observer, by means of a small hand-wheel, pulleys and a cord, is enabled to adjust the object while observing it, and instructed to keep it just at the limit of visibility, that is to make the lines gradually smaller while they are visible, and to increase their width while invisible. The movements of the test-object thus brought about are recorded on a kymograph drum by means of a lever and writing point. At the beginning or at the close of the experiment the abscissa lines are drawn in by setting the test-object successively at even millimeters and giving the drum one revolution. To get the mean setting of the instrument for any period it is necessary to scale the ordinate at each time-mark (every 5 seconds of the period) and compute the setting of the instrument corresponding to the mean ordinate.

The changes in the eye-aperture are brought about by using small diaphragms of blackened brass, as close as possible to the observing eye. The distance from the eye to the plane of the test-object is 125 cm. and the diameter of the circular field of the latter 3.5 cm. The distance from the diaphragm to the pupil of the eye is perhaps 1.5 cm. so that the divergence of the light-rays between the artificial and natural pupils is in the neighborhood of $\frac{3.5}{125} \times 1.5$ cm. or about 0.4 mm. This applies to the light from the entire face of the test object. The light from any point on its surface forms a pencil whose divergence after leaving the diaphragm, nearly 125 cm. away, is proportional to the diameter of the latter and negligible in the short distance remaining before it enters the pupil of the eye. The diaphragm is then the aperture from which the theoretical resolving power of the system is to be computed, and there is assurance that the

portion of cornea and lens used does not exceed the diaphragm by more than 0.4 mm. in diameter.

In order that the diaphragm should always be centered in the visual axis certain precautions are used. The test-object is viewed against a milk glass illuminated from behind by a lamp on an enclosed track. The room is kept dark except for an electric wall light back of the observer, which by means of a rheostat he can dim to a point such that the surroundings of the bright test field are just visible. The opening of the diaphragm, close to the eye, then appears as a blurred dim circle, and the observer's head, supported by a forehead-and-chin support is adjusted so that the test field appears exactly in the center of the circle. The wall light is kept lit during the experiment so that the observer always has a check on the position of his eye. The right eye is always used, vision by the other being cut off by a small black cloth hanging before it.

Six diaphragms are used of openings 1, 1.4, 2, 2.8, 4, 5.6 mm.[2] in diameter respectively, each having therefore approximately double the area of the next smaller. It is obvious that a change in the size of the diaphragm changes the amount of light entering the eye, and consequently the brightness of the image. Further, it is well known that simple increase in the brightness of a test-

[2] In order to show that the artificial pupil was always the limiting aperture, photographs of the eyes used have been since taken by flash-light. A millimeter scale was placed beside the eye and its photograph used to measure that of the pupil. The test-object was set at its highest brightness (189 candles per square meter) and the wall light used as in the experiments. Two photographs of each eye were taken, one as soon as possible after entering the experiment-room, and another on another day, after 20 minutes spent in manipulating and viewing the test-object as in the experiment. The results gave the following pupillary diameters.

	At once	*After 20 minutes*
C	6.0—6.2	6.0—6.2
G	6.5—6.75	6.1
J	6.8—7.0	6.5—6.7

The uncertainties in this method of measurement are indicated. The pupil showed a tendency to take a smaller diameter in two cases as the eye dark-adapted. Evidently the conditions under which these measurements were taken (without the artificial pupil) let more light into the eye than any experimental condition used, and even the largest artificial pupil (5.6 mm.) was unquestionably the limiting aperture in the experiments.

object increases the power of the retina in the perception of its detail. It is, therefore, necessary if we wish to gain knowledge of the character of the retinal image under varying apertures to eliminate differences of brightness of the retinal image. This is accomplished by a method about to be described as one detail of the experimental procedure.

Procedure

The 5.6 diaphragm is put in place. The observer, seated properly, begins to operate the test-object by means of the hand-wheel, and at a word from him the drum of the kymograph is started. He keeps the lines of the test-object as nearly as possible at the point of disappearance (or reappearance) and at the end of a minute the kymograph is stopped, the next smaller diaphragm substituted, a one-minute run taken and so on. When the smallest diaphragm is reached, a second run is taken with it, after a brief rest, and the series so repeated in reverse order.

Before each run, the lamp illuminating the test object is moved to such a point that the change in the amount of light entering the eye due to the change in the area of the diaphragm is balanced by an inversely proportional change in the brightness of the test-object. For example in changing from the 1.4 mm. diaphragm to the 1 mm. the illumination is increased as $(1)^2$ to $(1.4)^2$ or doubled, so that the retinal image of the test-object remains of the same brightness. This is practically accomplished by determining photometrically the positions of the lamp on the track which give illuminations as 1, 2, 4, 8, 16 and 32 and using each with its proper diaphragm.

The object of this detail of the procedure is to equalize all factors on which vision may depend other than those inherent in the refractive apparatus of the eye and dependent in magnitude upon the size of the aperture. Better vision results from simple increase in the amount of light, and if this were not compensated, might result from it alone when the aperture is increased. By diminishing the illumination of the test-object the retinal image is in each case brought to equal mean illumination, and a

difference in visual acuity can under such conditions be due only to unequal perfectness of the respective images.

With each observer, two series were taken as described, and two more without compensatory change of the illumination with the lamp at the nearest and farthest positions respectively. This latter gave the test field an average brightness of 5.92 candles per square meter measured photometrically, and the former consequently 32 times this or 189 candles per square meter.[3]

Results and Discussion

The detailed results from the three observers are given in the table together with the mean of the results for each set of conditions. These latter are embodied in the curves in figure 1.

From the table it will be seen that of the three observers, C[4] yielded (with few exceptions) the lowest values for visual acuity and J the highest while G stood between the two. It is further to be noted that every series of results showed a maximum value at some particular diameter of aperture, and that diameters greater and less than this optimum in the same series both gave lower results. This is to be attributed to the predominance of the effects of optical defects in the case of the larger diaphragm, and of the diffraction effect in the case of the diaphragm smaller than the optimum. In support of this it is to be noted that the mean variations of the individual results of the three observers from the average of the three are less under like external conditions for the small apertures, and increase somewhat irregularly with increase in the latter. In going from larger to smaller apertures the individual differences in the various eyes, in respect to their refractive regularity, become less and less important

[3] A perfectly diffusing surface which reflects all the light cast upon it has a brightness of 1 candle per square meter when the illumination upon it is π meter-candles. The two brightnesses given would then be matched by such a surface under illuminations of 18.6 and 593 meter-candles respectively. Any other surface which absorbs a part of the light would require a correspondingly higher illumination upon it to present an equal brightness.

[4] C wore correcting glass, -0.25 D sph. $+$ 1.75 D Cyl. Axis at 90°. The other observers required no correction.

and the diffraction effect, dependent alone on the size of the artificial pupil used and hence equal for all the eyes, increases and finally predominates in determining the limit of visual acuity.

The Relation between Visual Acuity and Size of Artificial Pupil

BRIGHTNESS CANDLES PER SQUARE METER	OBSERVER	APERTURE MILLIMETERS											
		5.6	M.V.	4	M.V.	2.8	M.V.	2	M.V.	1.4	M.V.	1	M.V.
I	C	5.06	0.27	5.28	0.11	5.44	0.25	*5.85*	0.14	4.88	0.21	3.95	0.04
	G	5.62	0.72	*5.83*	0.31	*5.69*	0.27	*5.81*	0.27	4.89	0.29	3.89	0.10
Compensated	J	6.70	0.16	*7.06*	0.21	*6.99*	0.09	6.48	0.14	5.31	0.06	4.09	0.04
	Mean	5.79		*6.06*		*6.04*		*6.05*		5.03		3.98	
	M. V.	0.60		0.67		0.63		0.29		0.19		0.08	
II	C	5.42	0.11	6.15	0.03	*6.44*	0.10	6.23	0.15	5.24	0.04	4.02	0.05
189	G	*7.08*	0.04	*7.14*	0.02	6.70	0.28	6.49	0.12	5.29	0.09	4.08	0.02
	J	*8.10*	0.18	*8.25*	0.08	8.06	0.08	7.17	0.21	5.44	0.06	3.98	0.03
	Mean	6.87		*7.18*		7.07		6.63		5.32		4.03	
	M. V.	0.96		0.71		0.66		0.36		0.08		0.04	
III	C	4.75	0.17	5.01	0.29	*5.35*	0.15	4.32	0.16	4.19	0.30	3.41	0.09
5.92	G	5.34	0.29	*5.77*	0.21	*5.76*	0.16	5.26	0.30	4.56	0.08	3.61	0.06
	J	7.10	0.11	*7.48*	0.05	6.90	0.27	6.13	0.39	4.57	0.28	3.55	0.02
	Mean	5.73		*6.09*		6.00		5.24		4.44		3.52	
	M. V.	0.91		0.93		0.60		0.61		0.17		0.08	

The values for visual acuity here given are in terms of the instrument reading. They may be translated into Snellen units by multiplying by 0.231.[5]

The results in Division I of the table are the means of four one-minute runs for each observer, in Divisions II and III two. The columns headed "M. V." give the mean variations of the separate results from the mean for each case. Similarly, the final results from the separate observers under identical conditions have been averaged and their mean variations given in the horizontal rows also designated "M. V."

These latter, therefore, measure the divergence of results between observers, the former the divergence of the individual observer's results.

It is interesting in this connection to compute the resolving power of the eye with the smallest diaphragm used and compare

[5] This comparison is to be qualified, owing to the fact that in the test-object used the bright and dark lines are only maxima and minima in brightness, the transition from the one to the other being a gradual one. In the case of the usual test-characters the transition is of course abrupt, between the low uniform brightness of the black and the high uniform brightness of the white parts of the test-object.

it with the results actually obtained. From the formula $\theta = 1.22\frac{\lambda}{D}$ when $\lambda = 0.00057$ mm. (the brightest part of the spectrum) and D = 1 mm. θ (expressed in minutes) = 2.39. The angular separation of the bright lines, when the test object is set to read 4.00 is 2.16 minutes—showing the observers' eyes to give

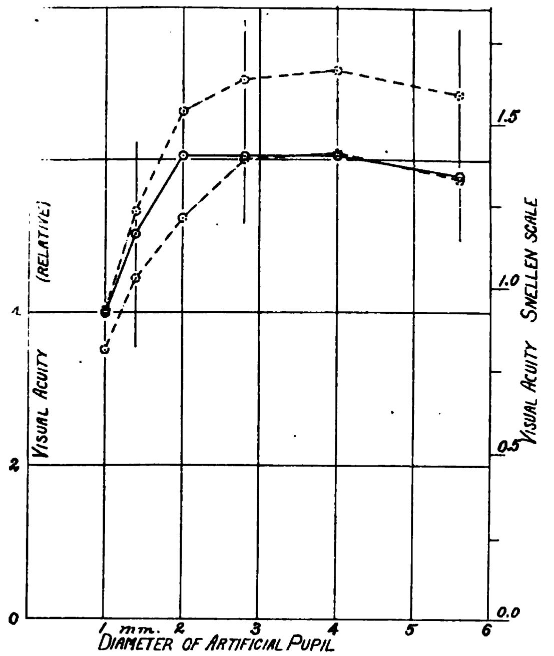

Fig. 1. The values plotted are the means for three observers. The solid line represents the values obtained with brightness compensated, the upper dotted line with constant brightness of 189 candles per square meter, the lower 5.92.

somewhat better vision than is called for by the formula. This is possibly because the latter is based on the smallest resolvable separation of two simple bright points, while the present experimental conditions have to do with an indefinite series of parallel bright lines which might favor the eye under an equal angular separation of the bright elements.

The optimal values for the pupillary diameter are indicated

by italicization of the results in the table. It will be seen that as a general rule, the eye which under equal conditions gives the best values for visual acuity, demands a larger pupil, other factors being equalized, than does an inferior eye to give the best result of which it is capable. Since *caeteris paribus* visual acuity increases with increase of brightness of the test-object, it is not surprising that where the brightness of the retinal image is allowed to increase with diameter of pupil (Divisions II and III of the table) the size of aperture for optimum vision is somewhat larger than it is when the test-object brightness is so compensated as to equalize the brightness of the retinal image (Division I).

Uhthoff obtained results mentioned above, with which the present ones are in agreement. On the other hand, Hummelsheim found increase in vision with decrease in pupillary diameter without exception. The fact that he did not compensate the illumination to obtain equal brightness of retinal images does not account for the discrepancy. As far as can be gathered from his work in the case of the observer showing the lowest visual an artificially contracted pupil (1.5 mm.), was 2.3 at 200 mk. illumination, and with the natural pupil (4 to 4.25 mm. at this illumination) 2.0. The present results show nothing but decrease of visual acuity with decrease in aperture between these limits. Attention is called here to the additional fact that in the present work visual acuity shows an absolutely lower value within this range, at brightnesses both above and below that just mentioned as used by Hummelsheim. If these latter were plotted in figure 1 the points would fall on the 4 mm. ordinate almost 40 per cent again as high as the present maximum (Snellen 1.68) and at a higher point as the zero ordinate is approached. This curve would be for a brightness of test-object intermediate between the two corresponding to the two dotted curves in the figure. This wide discrepancy is difficult to account for. It is to be borne in mind that the results of Uhthoff, obtained through technique similar to the writer's agree well with the latter. There are three possible reasons for the difference.

1. The fact that an artificial pupil allows a certain amount of divergence of the rays after passing through it, so that the area of the refracting surfaces of the eye so called into use is

somewhat in excess of the area of the artificial pupil. This would give the refractive errors of the eye greater weight than the case of a natural pupil of the same apparent diameter. However the difference due to this, as previously pointed out, is quite small.

2. The possibility in the present work that unknown to the observer his eye became, during the observation, somewhat decentered with reference to the axis of the system, so that in the case of the small diaphragm eccentric portions of the refracting surfaces of the eye came exclusively into play. This would tend to increase at least the chromatic error. There stands, however, the fact of the precautions used against this, and the further fact of the close agreement of all results obtained under like conditions in the present work (Divisions I and II of table, under 1 mm. aperture). This last point seems to rule out any error which could fluctuate enough to cause this wide discrepancy.

And 3, the fact that while Hummelsheim had his observers view the test character on a white card, with surroundings also illuminated and the immediate surroundings equal in brightness to the card, Uhthoff and the present writer used a test object on a background chiefly dark. Such a difference in external conditions undoubtedly affects the condition of the retina, although previous work (9) (10) has shown only a slight difference in visual acuity due to dark or bright surroundings, unless the latter are increased to a brightness considerably in excess of that of the test-object. This was not the case in Hummelsheim's method, and further, such a condition (excessively bright surroundings) always results in lower visual acuity, not higher.

The disagreement between his results and the present ones still wants explanation.

Summary of Conclusions

By the use of circular diaphragms before the eye it is shown that an aperture for optimal visual acuity exists somewhere between the limits 1 and 5.6 mm. for brightness of test-object from 5.9 and 189 candles per square meter.

When the illumination of the test object is compensated for the size of aperture to give equally bright images upon the retina

the optimum is somewhat less than when constant illumination is used and the brightness of the retinal image varies with the pupillary area. In the former case it falls at 2 to 4 mm., in the latter at 4 mm. on the average. Similar differences are plainly shown in the case of each of the observers.

Those observers showing on the whole better vision show also a larger optimal pupil.

With an aperture of 1 mm. diameter the several observers give almost identical results, which agree closely with the value of visual acuity calculated from the physical formula for the resolving power of the eye. Above this diameter the refractive errors of the eye and possibly also the limiting capacity of the retina itself come into play, and visual acuity, although at first increasing with increase of aperture, always fails to keep pace with the value computed from the formula.

The optimal pupil corresponds on the whole with the size of pupil accepted as normal for all except extreme conditions, namely, 2.8 to 4 mm. From this lower limit up to 5.6 mm. the variations in visual acuity with size of aperture are not large enough to be of practical consequence.

The writer wishes in conclusion to express the thanks due to his colleague, Dr. H. M. Johnson and to Mr. George Hathaway, technical assistant, for their coöperation and indispensable services as observers in this work, and to Mr. Albert Scheel and Mr. Roy Kerslake for the labor of computing the results.

BIBLIOGRAPHY

(1) Schuster, A. An Introduction to the Theory of Optics, London, 1909, pp. 144–149.

(2) Wood, R. W. Physical Optics, New York, 1911, p. 238.

(3) Drude, P. Lehrbuch der Optick, Leipzig, 1912, (III Auflage), p. 85.

(4) Uhthoff, W. Graefe's Archiv für Ophthalmologie, xxxvi, (1), p. 40, 1890.

(5) Hummelsheim, E. Graefe's Archiv für Ophthalmologie, xlv, 1898, pp. 357 to 373.

(6) Cobb, P. W. American Journal of Physiology, xxix, 1911, pp. 76–99.

(7) Ives, H. E. Electrical World, lv, 1910, p. 939.

(8) Johnson, H. M. Journal of Animal Behavior, iv, no. 4, 1914.

(9) Cobb, P. W., and Geissler, L. R. Psychological Review, xx, 1913, pp. 438 and 440.

(10) Cobb, P. W. ibid., xxi, 1914, pp. 24 and 27.

THE EFFECT OF REPEATED INJECTIONS OF PITUITRINE ON MILK SECRETION

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It is well known that the subcutaneous, intramuscular or intravenous injection of pituitary extract into lactating animals, man included, leads to an immediate increase in the amount of milk secreted by the mammary gland, abnormally rich in fat. This fact would at once suggest a practical method of increasing the output of milk in dairy cattle or milch goats, but it is found that there is a diminution in the yield at the next milking period, so that for the twenty-four hours the total quantity of milk or cream is not increased. There would appear to be another reason against the habitual use of pituitrine as a galactogogue, viz., that immunity to its action on the mammary gland is established. This, at any rate, we find to be the case in some of the goats with which we have been experimenting, and the observation we deem worthy of being put on record.

In our experiments on milk secretion in the goat we have used one animal for pituitary injection, at various periods extending over one year, and have found that the extract appears to become less and less effective as time goes on. This goat, when she came into our possession (October 31, 1913), was in a late stage of lactation, having given birth to her last kid in the end of March or beginning of April of the same year. On January 27, 1914, another kid was born. Neither of the kids was suckling when we made our experiments.

In the subjoined table (1) will be seen the effect of the injections (2 to 3 cc. of Parke, Davis and Company's pituitrine) on the different dates. The goat was milked dry immediately before

injection and again fifteen minutes after. The last section of the table (beginning July 14, 1914) gives the quantities obtained, under the same conditions, when no injection was given, i.e., the animal was milked dry and then fifteen minutes later milked dry again.

TABLE 1

Goat I

1913	MILK IN CC. BEFORE INJECTION	MILK IN CC. AFTER INJECTION	INCREASE PER CENT	FAT PER CENT BEFORE INJECTION	FAT PER CENT AFTER INJECTION
Nov. 27.........	50	40	80	12.9	18.0
Dec. 6.........	33	18	55	6.7	12.6
Dec. 7.........	35	18	51	6.3	10.2
Kid born January 27, 1914					
1914					
July 13.........	40	5	12	7.8	9.6
Oct. 1.........	28	2	7	8.3	
Oct. 16.........	25	4	16	9.5	
Oct. 20.........	20	2	10	9.7	
Control milkings without injection					
1914					
July 14.........	45	4	9	7.8	10.0
Oct. 2.........	30	1	3	8.2	
Oct. 3.........	25	1	4		
Oct. 17.........	20	1	5	8.6	
Oct. 19.........	20	2	10	9.1	

The next table (2) shows the results of the injection in the same animal (Goat I) made under somewhat different conditions. At 5 p.m. the pituitrine was injected without previous milking and fifteen minutes later the udder was milked dry. The amount of milk yielded at the same hour on the evening before, without injection, is taken as the control.

In another series of experiments, made for a different purpose, we were able to compare the effects on two goats, one (Goat I) which had been used in this work for several months and the other (Goat II) which had been in our possession for only a few days. Goat II, an old animal, was giving more milk at the time than Goat I.

Each was milked dry at 9 a.m. and again at 4 p.m.; at 6 p.m. the pituitrine was injected and fifteen minutes later the udder was emptied again. In table 3 the first column shows the milk in cubic centimeters yielded at 4 p.m. and the second at 6.15 p.m., after injection, for each goat.

Comparing the numbers on the control days with those on the days of injection, in the case of Goat I there is very little difference, whereas in Goat II the contrast is marked. On June 5 only 20 cc. of milk had accumulated in the gland between the hours of 4 p.m. and 6 p.m., while on the day following, in the

TABLE 2

Goat I

1913		AMOUNT OF MILK IN CC.	INCREASE IN CC.	INCREASE PER CENT	PERCENTAGE OF FAT
Nov. 19	No injection	58			
Nov. 20	Injection	88	30	52	
Nov. 23	No injection	75			8.9
Nov. 24	Injection	90	15	20	11.4
Second lactation period					
1914					
June 3	No injection	40			7.4
June 4	Injection	40	0	0	8.4
July 9	No injection	40			8.0
July 10	Injection	40	0	0	7.7

same two-hour period, under the influence of pituitrine, 100 cc. was obtained.

In a third goat the same effect is apparent. This was a young animal in her first lactation period. A kid was born on June 26, 1914, and killed by dogs on July 1. The mother came into our possession on July 3 after which she was milked by hand regularly, morning and evening.

On the dates given in table 4 this animal was milked dry at 6 p.m., injected, and again milked 15 minutes later. On alternate days the same procedure was adopted except that no pituitrine was given; the results are recorded in the second part of the table.

TABLE 3

1914	GOAT I				GOAT II			
	Milk in cc.		Fat per cent		Milk in cc.		Fat per cent	
	4 p.m.	6.15 p.m.	4 p.m.	6.15 p.m.	4 p.m.	6.15 p.m.	4 p.m.	6.15 p.m.
June 6	40	11	6.5		90	100	7.8	10.5
June 8	35	15	9.2	8.0	75	90	6.6	11.9
June 10	25	15	7.8	6.4	80	70	8.0	10.0
Control on days when no pituitrine was given								
June 5	45	8	7.6	5.4	100	20	5.8	7.3
June 7	45	14	8.8	8.2	95	22	5.9	5.8
June 11	40	15	8.3	8.4	85	25	6.5	4.9

A glance at the figures given above will show that the mammary gland appears to become less and less susceptible to the influence of pituitary extract the longer the administration is continued. This applies both to the quantity of milk yielded and to its fat content. The effect is best seen in the case of Goat I but it is also apparent in Goat III.

TABLE 4

Goat III

1914	MILK IN CC.		INCREASE PER CENT	FAT PER CENT	
	Before injection	After injection		Before injection	After injection
July 10	110	60	55	8.0	9.6
July 13	170	35	21	6.8	9.8
Oct. 1	70	10	14	8.7	10.0
Oct. 16	70	10	14	7.7	9.8
Oct. 20	70	7	10	9.7	8.0
Control milkings without injection					
July 14	200	5	2.5	7.2	10.8
Oct. 2	70	5	7	6.4	
Oct. 3	55	6	10	6.4	10.0
Oct. 17	70	5	7	8.2	10.4
Oct. 19	75	4	5	9.0	

Hammond finds that a goat, in the early stages of lactation, is more sensitive to small doses than one in a later stage, but our doses were all maximal, that is, 2 cc. or more. The stage of lactation cannot be the sole influence since in tables 1 and 2 both sets of observations were made about eight months after parturition, but in different lactation periods.

If it be a fact that immunity is established, then the pituitary secretion cannot act as a stimulant for the mammary gland in normal conditions.

SUMMARY

The administration of pituitary extract, by intravenous, intramuscular or subcutaneous injection, to a lactating animal leads to a marked increase in the quantity of milk secreted and also in its fat content. In the goat, if the injection be continued at intervals over a prolonged period—several months—immunity to its action on the mammary glands appears to be established both in regard to the amount of milk yielded and the percentage of fat it contains.

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CONTENTS

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VOL. 36 MARCH 1, 1915 No. 4

PROCEEDINGS OF THE AMERICAN PHYSIOLOGICAL SOCIETY

TWENTY-SEVENTH ANNUAL MEETING

St. Louis, December 28, 29, 30, 1914

Distribution of gastrin in the body. R. W. KEETOM and F. C. KOCH.

The gastrin extracts were obtained by adding 5 parts 0.4 per cent hydrochloric acid to one part tissue, heating to steam bath temperature, allowing to cool to and then to digest at room temperature for 24 hours. After filtering and concentrating the filtrate under diminished pressure, the proteins were removed by the addition of alcohol, the filtrate again concentrated under diminished pressure and extracted with hot absolute alcohol to remove vaso-dilatins. The insoluble residue was dissolved in water and diluted so that 1 cc. of the final solution represented 4 to 5 grams fresh tissue.

The preparations were injected intramuscularly into gastric fistula and Pawlow stomach dogs. These methods showed gastrin to be uniformly distributed in the pyloric and fundus portions of the stomach while in the cardiac area slightly less active products were obtained. Gastrin was found in lower concentrations in duodenal mucosa and in traces in the oesophagus. Smooth muscle, pancreas and submaxillary gland by the same methods gave inactive preparations. Brain tissue gave an extract causing slight increase in the volume of juice with the same acidity, but with a decrease in peptic activity. The results lead the authors to believe that they are dealing with a specific substance found in the gastro-intestinal tract, but localized chiefly in the stomach. This substance gives rise to a true secretion in contra-distinction to the brain extracts which probably cause vaso-dilation only.

The relation of the hunger contractions of the stomach to the normal digestion movements. F. T. ROGERS and L. L. HARDT.

Light on this question was sought in two ways; first graphic registration by the rubber balloon method of the activities of the stomach continuously from a time just after a meal until the hungry contractions were felt; second, X-ray observation of the hunger contractions using a bismuth-coated balloon with simultaneous graphic registration of the contractions. The first work was carried on with man and dogs, the second with dogs.

With the first method we find that there is present in the cardiac end of the stomach, in the absence of inhibiting nervous influences, a slow weak tonus rhythm which as digestion proceeds and the stomach empties itself becomes stronger and more rapid, culminating in the hunger contractions of the empty or nearly empty stomach. This tonus rhythm may be seen during the first hour after a meal. It is readily inhibited by nervous disturbances.

With the X-ray it may be seen that the weak hunger contractions are contractions of the fundus accompanied by a strong peristaltic wave which beginning near the cardia passes over the fundus. More vigorous hunger contractions are strong, rhythmical contractions of the whole fundus. If these contractions are peristaltic, their rate of advance is too rapid to be seen by this method. With the balloon in the pyloric end of the stomach only peristaltic contractions were seen.

The development of a tunicate without a nervous system. IDA H. HYDE.

From embryos and larvae of different ages of Ammaroecia, one of the Tunicates found in Woods Hole, up to date, five sets of experiments were made, controls kept, and material fixed and stained for further work.

The following is a preliminary résumé of the experiment and the results.

1. From free swimming larvae, or

2. From young embryos the tail or its anlage were removed. The organisms developed quite normally but were smaller than the control.

3. From free swimming larvae, or

4. From young embryos the tail and the nervous system or their anlage with the sense organs were removed. The results were small abnormal organisms, in which the heart began to beat and the siphons contracted slowly and incompletely. The investigation had not proceeded far enough to enable me to state that the heart reversed its rhythm spontaneously.

5. From embryos the nervous system in part or whole and the heart anlage were removed.

The resulting cell complex developed into an abnormal structure. The heart siphons and part of the digestive tract were missing. The cells that were left uninjured were capable of continuing their growth in a suitable environment up to a certain degree.

The influence of light on reproduction in Vorticella. IDA H. HYDE and CHRISTINE SPREIER.

Encysted Vorticellae were transferred by means of a Barber capillary pipette to a hanging drop infusion. When the zooids emerged from the cysts they were placed in a drop of the culture media on a hemocytometer micrometer slide. This was kept in a moist chamber. The Vorticellae of each series of the same age, were kept under the same conditions of temperature, moisture, food, and intensity of light but under different colored rays, of different degrees of intensity for at least three days. Colored glass, gelatin films and rays reflected from pure monochromatic colored cards both in bright and dim light were employed in studying the influence of colored light. For studying the influence of the intensity of light the Vorticellae were placed at different distances from the source of sun or electric light or back of smoked glass covers. The heat rays were cut out by water placed in parallel sided glass vessels in the path of the beams of light. From the results obtained we concluded that:.

1. Vorticellae exposed to daylight increased more rapidly in number on bright sunny days than on dark cloudy ones.

2. On an average 25 developed one-half meter, 10 one meter from the source of light and 2 in the dark, from one Vorticellae in 24 hours.

3. The average increase in bright light was 40 from green, 29 under yellow, 26 under white, 17 under red and 13 under blue.

In dim light it was 5 for yellow, 3 for green, 2 each for white, blue and red, from one Vorticellae in 24 hours.

4. We conclude that the stimulating effect on the reproductive power of Vorticella increases up to an optimum intensity of light rays, with the intensity of the light and that the bright luminous rays of yellow and green are more effective than the red and blue ones.

The relation between the erythrocytes and the haemoglobin to the oxygen tension of the respired air. H. C. DALLWIG, A. C. KOLLS, and A. S. LOEVENHART.

The objects of the work:

1. To further elucidate the proposition that decreased oxidation leads initially to stimulation.

2. To determine definitely the cause of the increases in erythrocytes and haemoglobin at high altitude.

3. To determine the relative susceptibility of the respiratory center and the red bone marrow to decreased oxidation.

4. To form a part of a general study of the relation of oxidation to functional activity.

Methods:

First. We have worked at atmospheric pressure keeping the animals in an atmosphere of oxygen and nitrogen of definite and constant composition but varying the concentration of oxygen and nitrogen in different experiments.

Second. We have kept animals in rarified atmospheres, the degree of evacuation of the chambers yielding the same oxygen tension as in the experiments at atmospheric pressure.

The animals used were dogs, guinea-pigs and principally rabbits.

Results:

1. Lessening of the oxygen tension in the respired air leads to an increase in the erythrocytes and haemoglobin.

2. This increase is due to increased activity of the red bone marrow as is shown by a microscopical study of the bone marrow, by the occurrence of a large number of basophilic macrocytes in the blood and by the fact that the haemoglobin and erythrocytes do not increase proportionately.

3. The changes occur only if the oxygen tension of the respired air is reduced below 14 per cent of an atmosphere. It was found by Haldane and Priestley that when the oxygen tension of the respired air falls to about 13 per cent, the respiratory center is stimulated by oxygen want. Hence the respiratory center and the red bone marrow are apparently equally sensitive to oxygen want.

4. It is immaterial whether the lowering of the oxygen tension is brought about at atmospheric pressure by keeping the animals in an atmosphere poor in oxygen and rich in nitrogen or whether the oxygen tension of the respired air is lowered by a partial evacuation of the respiratory chamber.

5. The carbon dioxide tension in the respired air is a factor of but small or no importance.

6. The increase in erythrocytes and haemoglobin occurs in animals which are naturally anaemic as well as in animals with a high blood count.

7. The increase in the haemoglobin and erythrocytes occurs only after three to seven days and the return to the normal blood count is slow and gradual, often requiring two months.

The comparative rate of the oxidation of enzymes and their corresponding pro-enzymes. W. E. Burge.

The object of this investigation was to determine if there was any difference in the rate at which pepsin and pepsinogen, trypsin and trypsinogen, respectively, were destroyed by oxidation.

The solutions of pepsin and pepsinogen were practically identical in strength after the activation of the pepsinogen. 150 coulombs of electricity were passed through 5 cc. of the solution of pepsin. Polarization was prevented by shaking the electrolytic cylinder. The amount of oxygen liberated by the passage of this amount of current was sufficient to oxidize practically all of the pepsin. The same amount of current was passed through an equal quantity of pepsinogen solution. The peptic activity of this solution was decreased by about 20 per cent. The conclusion is drawn that pepsinogen is more resistant to oxidation than pepsin.

The trypsinogen in 5 cc. of pancreatic juice was converted into trypsin by the addition of 2 drops of enterokinase. The amount of oxygen liberated by the passage of 150 coulombs of electricity through this solution practically destroyed its activity. A similar amount of electricity was passed through 5 cc. of pancreatic juice in which the trypsinogen had not been activated. Upon subsequently activating this electrolyzed solution it was found that the oxygen liberated by the passage of this amount of current had decreased its tryptic activity about 75 per cent. The conclusion is drawn that trypsin is more easily oxidized than trypsinogen.

On the concentration of sodium chloride in the plasma and its relation to the rate of excretion in normal and diabetic man. Franklin C. McLean.

The numerical laws governing the rate of excretion of sodium chloride formulated by Ambard and Weill (*Jour. de Phys. et Path. Gen.*, 1912,

vol. xiv) have been confirmed by us in numerous observations on normal individuals. The normal threshold for sodium chloride is 5.62 grams per liter of plasma, and when the concentration falls below this point excretion no longer occurs. Above this point the rate of excretion varies directly as the square of the excess of sodium chloride above 5.62 grams per liter. The excess may be calculated from the rate of excretion by the following formula, which corrects for the variable factors of the weight of the individual and the concentration of sodium chloride in the urine, as Ambard and Weill have shown that when the concentration in the plasma remains the same, the rate of excretion varies inversely as the square root of the concentration in the urine.

$$\text{Excess over } 5.62\ (\epsilon) = \sqrt{\frac{D \times \sqrt{\frac{C}{14}} \times \frac{70}{Wt}}{79.33}}$$

D = daily output, in grams
C = concentration of NaCl in urine, grams per liter
Wt = weight of individual, in kilos

The limit of error in applying this formula in normal individuals is about 0.08 gram per liter plasma, as determined by direct experiment in many individuals.

In eighteen diabetic patients examined about half were found to have a normal excretion of sodium chloride, as determined by comparison of the concentration of the plasma with the rate of excretion by the above formula. With one exception, a patient who had also a severe nephritis with chloride retention, the remainder of the cases were excreting sodium chloride on a markedly diminished threshold. In some of the cases the amount found in the plasma differed from the amount calculated by as much as 0.75 gram per liter. These patients complained of salt hunger, apparently due to the low chloride content of the plasma. In one case edema occurred while the patient was on a carbohydrate-free diet, and was apparently unaccompanied by any kidney change. Chloride retention in the plasma occurred during this edema, and the condition returned to its former state on disappearance of the edema.

When the intake and output of sodium chloride were measured in individuals excreting chloride on a lowered threshold the output was found to be normal, though much diminished during the occurrence of edema.

The diastases of the blood. HUGH McGUIGAN, and C. L. v. HESS.

If, as has been suggested as probable by ourselves and reported as a fact by others, diastases will dialyse through collodion, it favors the opinion that the diastases are free in the blood and not in the form of a pro-enzyme. Careful experiments, however, show that diastases will not dialyse. Even when concentrated and with little colloidal admixture—as in saliva—no dialysis will take place through collodion in six or eight hours.

The injection of starch into the circulation causes an increase in the amount of blood sugar. This has been taken as evidence by some that diastase is present free in the blood. Against this opinion, amongst others, the following objections have been raised.

1. Many solutions cause hyperglycemia when so injected.

2. In most cases the amount of starch injected can not account for the persistent increase in the sugar.

3. The apparent enzymotic action may be the result of injury and consequent unnatural liberation of enzyme.

4. That the action is due to living cells and not enzymes.

In reply to these objections we may say that so far as we have found:

1. Colloidal solutions—unless they are sugar formers—reduce rather than increase the blood sugar, when injected intravenously.

2. That the amount of starch injected may account for the rise in sugar.

3. That the enzymotic action is not due to injury—because injection of water and other injurious agents does not increase the enzyme concentration of the blood, as measured by v. Hess' method, and old blood is less active than fresh, and becomes still less active with time.

4. This we can not answer satisfactorily but think it is not cell action because the starch paste would first have to enter the cell, which is impermeable, and if once in and hydrolyzed, we know of no normal instance where the sugar is again excreted as such, but instead is used by the cell. The enzyme concentration of the liver is less than the blood, which indicates that the utilization of sugar is cellular while the hydrolysis of starch to sugar is enzymotic.

Essentials of v. Hess' method to compare the relative strength of diastase in solutions.

1. Solutions required. (1) A 1 per cent soluble starch solution which must be clear.

(2) A 0.002 per cent solution of iodine made when needed by dilution of a freshly prepared 1 per cent iodine in 3 per cent KI.

The determination should be made in triplicate at 37°C. as follows: Keep all solutions in a water bath and have temperature of all the same before mixing. Place 10 cc. of the starch paste in each of three equal sized test tubes. In each test tube leave a separate pipette which will give the same size of drop in each case. Place 1 cc. of the solution whose diastatic strength is to be determined in each of the test tubes. Keep the time exactly and test for the disappearance of the color by placing exactly 1 drop of the digesting mixture on a white test plate and add to it exactly 1 cc. of the iodine solution. Repeat every minute when near the end point. By this method, when carried out as directed, the relation of the iodine to the starch is not varied and very accurate results can be obtained. The diastatic content of the blood of the dog varies but very little from day to day. The end point is usually reached in about thirty-five minutes.

The action of pituitrin on the mammary gland. W. L. GAINES.

The action of pituitrin on the mammary gland was followed by observation of, first, the effect on milk secretion, and, second, the change in internal volume of the gland. In the volume method a cannula was inserted in the teat of a goat, the gland inflated with air and connected with a manometer recording on a kymograph.

Upon intravenous injection of pituitrin the active gland, milked dry by hand, shows an abrupt decrease in volume and increase in pressure, followed by a gradual return to the initial volume and pressure. A repeat dose gives a similar result. This repetition excludes milk secretion as a possible cause of the volume and pressure changes, since a repeat dose causes no secretion of milk. The non-lactating gland shows no response on injection of pituitrin, and this is true, also, when the other gland of the pair is still functional. The absence of any response in the inactive gland excludes vaso-motor changes as a possible cause of the results observed in the case of the active gland. Pituitrin causes a contraction of the gland musculature when the gland is functional, but has no effect when the gland is inactive, and the difference in sensitiveness of the gland musculature in the active and inactive gland is not due to the presence or absence of any particular substance in the circulating blood stream.

In normal nursing of the dog the musculature of the gland is involved and is excited reflexly by the nursing of the pups, so that the rate of secretion is at first slow, increasing to very fast, then decreasing to zero. At the zero stage pituitrin causes no further secretion. The reflex

mentioned is partially or completely inhibited by ether anesthesia, so that the nursing pups are able to secure only a small portion or none of the normal yield, and in this condition injection of pituitrin causes an immediate secretion of milk, to equal the normal yield. A repeat dose causes no further secretion.

In the goat hand milking at equal time intervals gives normally a fluctuating yield. Injection of pituitrin immediately following a low yield causes a marked further secretion; but, following a high yield it causes little or no further secretion. The effect of pituitrin on milk secretion upon injection immediately following natural nursing or artificial milking is dependent on the efficiency with which the muscular mechanism of the gland has been brought into play by the nursing or milking to remove the accumulated milk from the gland.

The influence of depancreatization upon the state of glycaemia following the intravenous injection of dextrose in dogs. I. S. KLEINER and S. J. MELTZER.

In former experiments (see *Proceedings of the American Physiological Society*, vol. 33, 1913, p. xxvii) it has been shown that after the intravenous injection of large amounts of dextrose (4 g. per kilo) into dogs the sugar rapidly disappears from the blood stream so that after 1½ hours after the end of the injection the blood-sugar falls nearly to its original figure. In the present experiments the same procedure was carried out on completely depancreatized dogs. In these cases the blood-sugar did not fall to its original value or near it; at the end of 1½ hours it was on the average more than twice as high. The following is a comparison of the average figures:

	BLOOD-SUGAR			
	Before injection	End of injection	1½ hours after end of injection	Dextrose in urine, % of amount injected
Normal (5)	0.20	0.79	0.27	> 43
Depancreatized (9)	0.38	1.19	0.86	49 (uncorrected for "diabetic" sugar).

A similar difference was observed also in nephrectomized dogs.

It is claimed by some investigators that the glycaemia following depancreatization is due to an over-production of sugar. It is evident that the hyperglycaemia in our cases of depancreatization can not be due to such a factor. We shall not discuss here whether our results

can be adequately explained by the assumption that the removal of the pancreas causes a decrease in the consumption of dextrose by the body tissues. We wish, however, to indicate that some of our facts hint at the possibility of a change in the permeability of the endothelia of the circulatory apparatus as a factor in the results of depancreatization.

Recuperation. Nitrogen metabolism of a man when ingesting successively a non-protein and normal diet after a seven-day fast. F. D. ZEMAN, JEROME KOHN and PAUL E. HOWE.

This is the third[1] of a series of experiments concerned with changes in metabolism of man following the ingestion of food after a fast. In the recuperation periods (4 day) of this experiment non-protein and normal diets were fed; the preliminary and final diets were the same. The non-protein diet consisted of cane sugar, clarified butter, an alkaline salt mixture and agar agar having an approximate daily fuel value of 3500 cal. Determinations were made of the body weight, and the excretion of water, total N, urea, ammonia, creatine and creatinine in the urine.[2]

The excretion of the various urinary constituents followed the usual course during the fast; the total N excretion on the seventh day was approximately 10 grams and creatine appeared on each day. The ingestion of a calorifically sufficient non-protein diet resulted in a decrease of the nitrogen excretion which became constant on the third and fourth days. Minimum values obtained on the second day of feeding, were as follows: total N 3.56 grams, urea-N 1.59 gram, ammonia-N 0.54 gram, creatinine-N 0.61 gram, creatine-N 0.05 gram. A relatively high ammonia-N excretion (0.72 gram, 17.4 per cent. of the total N) occurred on the third day. Normal conditions tended to return in the final period while the subject was retaining nitrogen. A lowered absolute and relative ammonia-N excretion was observed.

The daily nitrogen excretion through the feces during the non-protein period was 0.50 gram.

A comparison of the changes in body weight and the nitrogen balances shows an increase in body weight during the non-protein feeding period accompanied by a loss of nitrogen while the reverse occurred in the final period. The initial increase in weight upon the ingestion of food is the

[1] The first two experiments were reported by Howe, Mattill and Hawk, Jour. Amer. Chem. Soc., 33, p. 568, 1911, and Howe and Hawk, Proc. Amer. Soc. Biol. Chem., 2, p. 65, Jour. Biol. Chem., 11, p. xxxi, 1912.

[2] Variations in factors associated with changes in the urinary acidity are reported in another connection.

result chiefly, of the retention of water and to a smaller degree of non-nitrogenous food substances.

Apnoea as an after-effect of pulmonary distention, and its dependence upon the vagus nerves. T. S. GITHENS and S. J. MELTZER.

In recent years the conception became dominant, due especially to the investigations of Haldane and his pupils, that apnoea as an after-effect of distension of the lung is essentially of chemical origin, due to a reduction of carbon dioxid in the blood circulating through the respiratory center; this has been designated by them as "true apnoea," Furthermore, it was recently stated that there is no experimental evidence for a possible claim that true apnoea *could* depend exclusively upon the intactness of the vagus nerves.

Of our recent investigations of this subject we wish to mention here the following three facts observed by us (and recently demonstrated at a meeting of the Society for Experimental Biology and Medicine). 1. A fairly prolonged characteristic apnoea follows a short distension of the lungs in dogs *without any previous artificial respiration.* The duration of the apnoea depends, within certain limits, upon the degree of pressure used for the distension (Meltzer's pleural cannula was used for the graphic presentation of respiration). 2. The same apnoea after-effect can be obtained when air used for distention of the lungs contains 5 per cent CO_2. 3. No such apnoea after-affect can be obtained after both vagus nerves are cut.

These experiments demonstrate that the mere distension of the nerve endings of the pulmonary vagus without the aid of a chemical factor (acapnia) is capable of producing a prolonged apnoea as an after-effect of the mechanical stimulus. The restriction of the term "true apnoea" to a condition produced exclusively by chemical changes does not seem to be well founded.

Experimental hyperthyroidism. W. B. CANNON, C. A. L. BINGER and R. FITZ.

Interest in the bodily changes during or following emotional excitement led us to enquire concerning the nature of certain diseases often reported as having emotional origin. We proceeded on the theory that repeated emotional experiences might lower a naturally high neurone threshold and thus result in frequent stimulation of parts which normally are only occasionally roused to special activity. To test the effect of over stimulation two of us (W. B. C. and C. A. L. B.) fused in the cat the anterior root of the right phrenic nerve with the right cervical

sympathetic cord. Thus after regeneration had occurred, there was delivered to neurones in the superior cervical ganglion a volley of impulses every time the animal breathed. The operations were performed early in May. In October four of six animals were still alive. All had peculiar symptoms. There was marked tachycardia—the average heart rate in 36 observations on normal cats was 165, in 30 observations on these animals it was 222. Though fed like normal animals they had loose movements of the bowels. They suffered from falling of the hair from the neck and back, and they acted as if afflicted with pruritus of the head and toes. They were unusually excitable, as indicated by rushing away when taken in hand or petted. One of us (R. F.) has studied the basal metabolism, and found that the average heat loss per kilo per 24 hours in normal adult cats is 44 calories; in three of the four experimental animals it was 66 calories,[1] and in one (in all ways the most profoundly altered animal) it was 112 calories—an increase over the normal of more than 150 per cent. This animal, after very rapid loss of weight, has died. At autopsy the adrenal glands were found nearly three times the average weight. In dim light the pupil in these animals was larger on the operated side, and in one of them exopthalmos and respiratory hippus have developed on that side. These symptoms are, in the main, characteristic of exophthalmic goiter, as seen in man.

The observations on these four animals is preliminary to a more extensive study of the subject. The method of using the phrenic as a source of stimuli is now being applied not only to the thyroid gland but also to other organs innervated by the autonomic system. We have planned a series of studies on overaction of the autonomic, to be carried out by use of this method.

Oxidation in the erythrocytes of the goose. J. F. McClendon.

The oxidative coloring of leucobases by erythrocytes was increased on laking, due to mixing of oxyhaemoglobin with the leucobase. (See my paper on oxyhaemoglobin below).

Colorimetric methods in general use for measuring intracellular oxidations were discarded, since oxyhaemoglobin was found to oxidise many of these substances. The erythrocytes (in Ringer) were shaken with air in a flask connected with a water manometer, and immersed in a thermostat. The oxygen absorbed was equal to the oxygen used by the cells (since the haemoglobin was saturated with O_2 at the beginning).

[1] The metabolism of one of the three has since gradually risen—77, 87, 98, 109, 103 calories—with corresponding loss of weight.

Numerous experiments, made under a great variety of conditions to determine whether induction shocks increase intracellular oxidations, failed to show such increase. If induction shocks affect the cells physiologically, it is probable that strong shocks affect them more than weak ones. It was found that the electric conductivity was slightly increased by strong shocks, without laking any of the cells. This suggests that one might obtain an increased coloring of a leucobase by erythrocytes subjected to the shocks, since the increased permeability might allow the leucobase to reach the oxyhaemoglobin more rapidly. Such a result was obtained by R. Lillie.

In the oxidation experiments, the manometer readings are greatly affected by changes in temperature and barometric pressure. In order to equalize these, a brass tube 2 *m.* long and 7 mm. bore was coiled in the thermostat and connected with a Marey's tambour, the platinum pointer of which, dipping in mercury, made and broke a heat-controlling electric circuit. Readings were made in this "barostat," whereas the oxidation took place in a thermostat independent of temperature. The "barostat" was sensitive to a thousandth of a degree, but might change 6° due to change in barometric pressure.

The increase in permeability of the frog's egg at the beginning of development as determined with the nephelometer. J. F. McClendon.

Three years ago, I observed that the unfertilized frog's egg could be made parthenogenetic by a momentary electric shock, and gave reasons for supposing that the electric shock (or the spermatozoon in normal fertilization) increased the permeability of the egg. Recently, I proved this supposition to be correct. The permeability of the unfertilized egg to NaCl was found to have increased on stimulating the egg with an electric shock (which caused it to begin normal development).

Several methods were tried for the quantitative estimation of sodium ions, but the results with such small quantities would not be considered trustworthy had they not tallied with the more certain results on the determination of chlorine ions with the nephelometer.

Lot 1 was stimulated by an electric shock from clean platinum electrodes (in about one minute all of the eggs had turned the black pole upward; 23 hours later first cleavage began) and lot 2 used as a control. Twenty cubic centimeters of H_2O were added to each lot and at the end of one hour this water was analyzed. There was more $Na+$ and $Cl-$ in the water from the stimulated eggs than in the control. Whether this increase in permeability is the cause of development has

not been determined. It is not restricted to the frog's egg, however, since I found the same true of the sea urchin's egg, a fact which has been confirmed by Gray, at Plymouth.

The unfertilized frog's egg placed in tap water or distilled water continues to swell until death ensues. This death is probably caused by the swelling and the latter by the osmotic pressure of the soluble substances contained within it. The increased permeability allows the escape of NaCl and lowers the internal osmotic pressure, thus retarding the swelling and preserving the life of the egg.

Some experiments on the oxidising power of oxyhaemoglobin. J. F. McClendon.

Many leucobases + H_2O_2 are colored on the addition of blood pigments, but some of these bases are oxidised on the addition of dog's erythrocytes, in the absence of H_2O_2, especially if the solution is slightly more alkaline than the blood. Alpha-napthol, aloin, or paraphenylendiamide is colored rapidly—benzidine or guaiac, not so. The same oxidising power is possessed by oxyhaemoglobin, recrystallised five times, so it is not due to so called "oxidases" as an impurity. Methaemoglobin recrystallised seven times, oxidised these substances.

Some measurements were made with the Dubosque colorimeter, on the rate of oxidation of Vernon's "substrate" ($\frac{1}{100}$ normal alpha napthol and paraphenylendiamide, slightly alkaline). Solutions of dog's erythrocytes, oxyhaemoglobin crystalized five times and methaemoglobin crystallized seven times were made up to be equivalent to 5 per cent of blood. These solutions were mixed with equal volumes of substrate and studied in pairs, with an arrangement to compensate for the difference in color of the blood pigments. The solution of erythrocytes did not oxidise the "substrate" faster than the oxyhaemoglobin or the methaemoglobin.

We thus see the necessity of removing the last traces of blood pigments in studying the so-called "oxidases" colorimetrically.

It is a recorded fact that blood charcoal oxidises oxalic acid to CO_2 and H_2O, whereas other charcoal is less effective, or not at all. This might be due to the presence of iron and adsorption surfaces. Since Warburg has shown that adsorption plays a rôle in the oxidative power of oxyhaemoglobin, we might expect that the blood pigment would behave differently inside and outside of the corpuscle, since it is too concentrated within the corpuscle to exist in simple aqueous solution and probably has a different aggregation state which would affect adsorption.

Lactic acid and sugars are not oxidised to CO_2 and H_2O, by blood charcoal or by oxyhaemoglobin.

The harmful effect of a vegetable diet. CARL VOEGTLIN.

Feeding experiments with natural vegetable foods in monkeys, white mice and rats, hogs, and fowls are reported with the following results: (1) An exclusive diet of cereals of good quality such as wheat, corn, barley, oats, millet, etc., is injurious to some mammalia and leads sooner or later to the death of these animals. (2) An exclusive diet of some fresh vegetables, such as carrots, Irish potatoes and sweet potatoes has the same effect. (3) Legumes such as beans and peas seem to be insufficient for the maintenance of life if forming the only diet of mice and rats. (4) Fresh beef, ox liver, eggs and milk, if added in sufficient amounts to the vegetable food will protect the health of these animals. (5) A mixed vegetable diet composed of cereals, legumes and fresh vegetables is inadequate for the maintenance of life in mice and certain other mammalia. (6) Fowls can live in perfect health for a long period on an exclusive diet of corn, wheat and other cereals. They die if put on an exclusive diet of corn oil cake meal (absence of certain vitamines). In those animals, which finally died as a result of an exclusive vegetable diet, symptoms were noticed pointing to a pathological condition of the central nervous system and the alimentary canal (paralysis, strychnine-like convulsions, diarrhoea or constipation). Marked histological changes were found in the organs of these animals. The addition of extracts of beef liver and yeast (vitamines) to the vegetable diet does not seem to have any effect on the occurrence of pathological symptoms and subsequent death of the animals. When certain inorganic salts (calcium and sodium phosphate) are added to the corn diet, the life of the animals (mice) is very much prolonged. Our present knowledge of the nutritive value of most of the natural vegetable foods is very limited. The reported experiments demonstrate the inadequate composition of these foods and the harmful effect they may produce in animals if fed exclusively. That a mixed diet composed of both vegetable and animal foods is undoubtedly less apt to be harmful under normal conditions is well demonstrated by everyday experience.

The path of conduction between the sino-auricular and the auriculo-ventricular nodes. J. A. E. EYSTER and W. J. MEEK.

All recent work seems to show that in the normal cardiac cycle electrical negativity is first to be observed in the sino-auricular node.

A few hundredths of a second later the same condition becomes manifest in the auriculo-ventricular node. The work here reported has been an attempt to find the path by which this wave of negativity passes from one node to the other. Our methods have been electrical and have consisted in determining by string galvanometers the actual and relative times at which various regions of the supraventricular parts entered into activity.

In the first series of experiments differential electrodes of a modified Clement type were placed on the S-A node, the body of the right auricle and on the A-V node. It was found that in many cases the A-V node became active before the body of the auricle and that in the cases in which the reverse was true the time interval was apt to be very short. In reversed rhythms the S-A node was often negative before the auricle. These results have been interpreted to mean that conduction between the two nodes is not usually at least by way of the right auricle.

In a second series of experiments a double circle of points around the S-A node were carefully compared with each other to find which first showed negativity. The rule is almost invariable that the venous side shows activity before the auricular. The region most often showing negativity soonest after the node itself is an area immediately adjoining the head of the node on the venous side.

In a third series the differential electrodes were placed as at first and then various cuts and ties were made around the S-A node. Interrupting tissue connections between the S-A node and the auricle always delayed sino-auricular conduction time but it had no other effect on the heart. Tieing the bundles of tissue which run off from the end of the sulcus terminalis was without effect. Each interruption of tissue on the venous side without exception delayed the conduction time between the S-A and A-V nodes. More than one cut was apt to produce auriculo-ventricular rhythm.

Our results seem to indicate that the path of least resistance between the upper and lower auricular regions of specialized tissue lies on the venous side of the S-A node and does not involve the body of the right auricle. So far as can be told at present this path is probably a diffuse one.

The metabolism of the resting nerve and its correlation with the direction and rate of nerve impulse. SHIRO TASHIRO.

There is a gradient of carbon dioxide production in the unstimulated nerve. This gradient of chemical condition is parallel to the direction

of the normal nerve impulse, and not to the direction of development of the fiber from the nerve cell. Many experiments made on various kinds of "pure" nerve fibers, including sensory dendrites, enable us to generalize this by saying that the normal nerve impulse, in the resting nerve, passes toward a point of lower carbon dioxide production.

There seems to be a close relation between the rate of nerve impulse and the production of carbon dioxide in the resting nerve, if one compares corresponding nerves from different animals. The data for such a generalization must necessarily be cumulative. The limited data we have secured indicate that the nerve which gives off more carbon dioxide in the resting state conducts the nerve impulse more quickly.[1]

There are several conditions which affect the rate of nerve conduction, e.g., temperature and change in concentration of electrolytes in a solution surrounding the nerve. Mayer found that the rate of nerve conduction in the subumbrellar regions of Medusa Cassiopeia increases about 5 per cent in sea water diluted with distilled water (9:1), while it decreases 50 per cent in 50 per cent sea water. By substituting 0.9 molecular dextrose for distilled water he demonstrated that the change in the rate of nerve impulse in diluted sea water is not due to the decrease in osmotic pressure, but to the change in concentration of the electrolytes. If, under these conditions which decrease the rate of the nerve impulse, a measurement of carbon dioxide production is made on a thin layer of regenerating ectoderm tissue before the muscle regenerates, we find that there is a parallelism between the rate of nerve conduction and carbon dioxide production.

The temperature coefficient of velocity of the nerve impulse is known to be greater than that of most purely physical processes. We find that the temperature effect on carbon dioxide production from non-stimulated nerves (the claw nerve of the Limulus) is of about the same magnitude as that of the velocity of the nerve impulse.[2]

These facts seem to indicate that there is a definite relation between the metabolism (as measured by carbon dioxide production) in the resting nerve and functional activity in the nerve fiber, including the direction and rate of the nerve impulse.

[1] The reason why these relations will not hold if we compare non-medullated with medullated fibers will be published later.

[2] I must add here that the temperature coefficient of the velocity of nerve impulse in the claw nerve of Limulus has not yet been worked out.

FEEDING EXPERIMENTS ON RATS

III. A Further Contribution to the Knowledge of Organs With an Internal Secretion

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A series of experiments is being carried on in this laboratory to study the influence upon growth and development of the various internally secreting glands. These glands are being fed to white rats in stated portions, at regular intervals.

The experiments have not proceeded far enough to make a detailed report on the effect of all the organs used. Here merely a preliminary account of some observations on the thyroid treated animals will be given.

Fresh beef thyroid was used and given in portions small enough to keep the animals in fairly good health. In the earlier experiments too large a dose of thyroid had been given, 5 g a week, so that the animals very soon showed all the well-known signs of hyperthyroidism usually leading to death. The dose was gradually cut down to 1 g a week, in other experiments to 1 g in 5 days. Even with such small doses the symptoms of hyperthyroidism were sometimes slightly noticeable, however, the animals kept so well that they were able to produce offspring. After the dose of thyroid had been cut down considerably, the animals not only bred, but the young were also strong enough to keep alive.

In the following list four matings will be described in detail, the offspring of which are still living.

I. ♂t × ♀t[1]

HISTORY OF FATHER

Born July 21, 1913.
Thyroid feeding began January 6, 1914.

1. Mating: ♂t × ♀t 1/26–2/16[2] 1914; died; no pregnancy.
2. Mating: ♂t × ♀t 2/19–3/10, 1914; ♀ died; no pregnancy.
3. Mating: ♂t × ♀t 3/21–5/15, 1914; no pregnancy.
   Thyroid feeding stopped 5/15, 1914.
4. Mating: ♂t × ♀n 5/15–6/8, 1914. 10 young 6/10. Frail, died within a week.

HISTORY OF MOTHER

Born December 31, 1914.
Thyroid feeding began 3/24, 1914.
Thyroid feeding stopped 6/10, 1914.

Final mating which gave living offspring

♂t × ♀t 6/12–7/6, 1914. 3 young born 7/9, 1914. Young are living now. They are much smaller (figs. 1, 2) than the normal rats of the same age (fig. 3). At the age of 152 days they weighed 97 g, 101 g, 190 g resp. Normal rats of this age weigh from 150–220 g.

SUMMARY OF CASE I

(a) While under treatment the father was bred unsuccessfully to 3 treated ♀.

(b) After discontinuation of the thyroid treatment the father was bred to a non-treated ♀. 10 young were born after 26 days, all so frail that they died within a week.

(c) One month after the thyroid treatment had stopped, the father was bred to the mother, the thyroid treatment of which ceased on the mating day. The results were 3 undersized young born 54 days after the father and 29 days after the mother had received the last dose of thyroid.

In this case father and mother received thyroid *before* mating.

II. ♂t × ♀n.

HISTORY OF FATHER

Born January 28, 1914.
Thyroid feeding began 3/24, 1914.
Thyroid feeding stopped 6/10, 1914.

HISTORY OF MOTHER

Born February 26, 1914.
Normal feeding.

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[1] t = treated. n = normal.

[2] 1/26–2/16 means: ♂ and ♀ were kept together from January 26 until February 16.

Final mating which gave living offspring

♂*t* × ♀*n* 6/12–7/6, 1914. 4 young born July 10, 1914. 2 young died after 3 days; 2 young living, undersized (figs. 4, 5).

SUMMARY OF CASE II

The father immediately after the thyroid treatment had ceased, was bred to the non-treated mother. 4 young were born 30 days after the last thyroid dose had been given. 2 young died soon, 2 undersized ones are living.

In this case the father only received thyroid *before* mating.

III. ♀*t* × ♂*n*.

HISTORY OF FATHER

Born December, 1913.
Normal feeding.

HISTORY OF MOTHER

Bought November, 1913, about 3 months old.
Thyroid feeding started 1/6, 1914.

1. Mating ♀*t* × ♂*t* 3/21–5/15, 1914; no pregnancy.
   Thyroid feeding stopped 5/15, 1914.

Final mating which gave living offspring

♀*t* × ♂*n* 5/15–9/6, 1914. 6 young born 9/6, 1914. 2 young die 10/13, 1914. 4 living, small size (figs. 6, 7, 8) control figure 9. At the age of 94 days they weighed 46, 50, 58, 83 g resp. Normal rats of this age weigh from 120–150 g.

SUMMARY OF CASE III

(a) While under treatment the mother was bred unsuccessfully to a treated ♂.

(b) The treated mother immediately after the thyroid treatment ceased was bred to the non-treated father. 6 young were born 113 days after the last thyroid dose had been given. 4 young, smaller than the normal, are living.

In this case the mother only received thyroid *before* mating. She required three months to recover from the thyroid influence.

IV. ♂*t* × ♀*n*.

HISTORY OF FATHER

Born July 24, 1913.
Thyroid feeding started 3/21, 1914.

1. Mating ♂*t* × ♀*t* 3/21–5/15, 1914; no pregnancy.
   Thyroid feeding stopped 5/15, 1914.

HISTORY OF MOTHER

Born May 26, 1914.
Normal feeding.

1. and 2. Matings: Bred to two normal males successively. 6 young and 8 young.

Final mating which gave living offspring

♂*t* × ♀*n* 5/15–9/10, 1914. 3 young born September 10, 1914. Undersized. At the age of 90 days they weighed: 52, 63, 99 g resp. Normal rats at this age weigh from 120–150 g.

SUMMARY OF CASE IV

(a) While under treatment the father was bred unsuccessfully to a treated ♀.
(b) The normal mother was bred successfully to 2 normal ♂.
(c) The treated father immediately after the thyroid treatment had ceased was bred to the normal mother.
3 young were born 117 days after the last thyroid dose was given.

In this case the father only received thyroid *before* mating. He required three months to recover from the thyroid influence.

To this list must be added a number of cases in which young were born alive, but died after puberty.

V. ♂*t* × ♀*n*.

HISTORY OF FATHER

Bought January, 1913. About 3 months old.
Thyroid feeding started January 13, 1913.
1. Mating: ♂*t* × ♀*t* 4/7–4/28, 1913; no pregnancy.

HISTORY OF MOTHER

Bought January, 1913. About 3 months old.
Normal feeding.

Final mating which gave offspring

♂*t* × ♀*n* 4/7–4/28, 1913. 5 young born 5/1, 1913.

HISTORY OF THE YOUNG

Born May 1, 1913.
1. Young died after 9 days. Very frail.
4 other young started on thyroid diet October 30, 1913. They were all undersized and rather feeble. One dragged the hind legs. When 84 days old they weighed: 57 g, 58 g, 60 g, 66 g, resp. Normal rats at this age weigh from 110–140 g.
2. Young died November 10, 1913.
3. Young died December 3, 1913.
4. Young died December 5, 1913.
5. Young died December 21, 1913.

SUMMARY OF CASE V

(a) While under treatment the father was bred successfully to a treated ♀.
(b) While under treatment the father was bred to the non-treated mother.
5 young were born 24 days after mating. 1 died 9 days old, 4 frail and undersized lived several months.

In this case the father only received thyroid up to the time of mating.

VI. ♂ t × ♀ t.

HISTORY OF FATHER

Bought January, 1913. About 3 months old.
Thyroid feeding started January 13, 1913.

1. Mating: ♂ t × ♀ t_2 4/7–4/28, 1913; no pregnancy.
2. Mating: ♂ t × ♀ n 4/7–4/28, 1913. 5 young 5/1, 1913 (case V).

Final mating which gave offspring

♂ t × ♀ t_2 5/26–9/14, 1913.
(Female in this successful mating is the same as in the first unsuccessful mating).
Thyroid feeding stopped June 26, 1913.

HISTORY OF MOTHER

Bought January, 1913. About 3 months old.
Thyroid feeding started January 13, 1913.

1. Mating: ♀ t × ♂ t_2 4/7–4/28, 1913; no pregnancy.

Final mating which gave offspring

♂ t × ♂ t_2 5/26–9/14, 1913.
Thyroid feeding stopped 6/26, 1913.

HISTORY OF THE YOUNG

7 young born September 14, 1913. Very frail and undersized. These young were started on thyroid October 31, 1913.

2 die November 10, 1913.
3 die November 12, 1913.
2 die November 15, 1913.

SUMMARY OF CASE VI

(a) While under treatment the father was bred unsuccessfully to the treated mother.

(b) While under treatment the father was bred to a non-treated ♀ (see case V).

(c) The treated father and treated mother (under a) were again mated. The thyroid treatment ceased 31 days after mating.

7 young were born 81 days after the last thyroid dose was given.

The treated parents required two months to recover from the thyroid influence.

VII. ♂ t × ♀ $\dot{n}$.

HISTORY OF FATHER

Born July 21, 1913.
Thyroid feeding started January 6, 1914.

1. Mating: ♂t × ♀t 1/26–2/16, 1914; no pregnancy.
2. Mating: ♂t × ♀t 2/19–3/10, 1914; no pregnancy.
3. Mating: ♂t × ♀t 3/21–5/15, 1914; no pregnancy.

Thyroid feeding stopped May 15, 1914.

Final mating which gave offspring

♂t × ♀n 5/15–6/9, 1914. 10 young born 6/10, 1914. All die during June.

SUMMARY OF CASE VII

(a) While under treatment the father was bred unsuccessfully to 3 treated ♀.

(b) The treated father, the thyroid treatment of which ceased on the mating day, was mated to the non-treated mother.

10 young were born 26 days after the last thyroid dose was given. All died within 2 weeks.

In this case the father only received thyroid *before* mating.

VIII. ♀t × ♂n.

HISTORY OF FATHER

Born May 26, 1913.

1 and 2. Matings: ♂ bred to 2 normal females successfully. 8 and 7 young.

HISTORY OF MOTHER

Born July 21, 1913.

1. Mating: Bred to a normal male. 6 young born 1/26, 1914. Thyroid feeding started March 21, 1914.
2. Mating: ♀t × ♂t 3/21–5/15, 1914; no pregnancy.
   Thyroid feeding stopped May 15, 1914.

Final mating which gave offspring

♀t × ♂n 5/15–10/14, 1914.

5 young born 10/14, 1914, all eaten up by 10/19, 1914.

SUMMARY OF CASE VIII

(a) The non-treated father was bred successfully to 2 non-treated ♀.

(b) Before thyroid treatment began the mother too was bred successfully to a non-treated ♂.

(c) While under treatment the mother was bred unsuccessfully to a treated ♂.

(d) Finally the treated mother, immediately after the thyroid treatment had ceased, was bred to the non-treated father.

5 young were born 151 days after the last thyroid dose was given.

In this case the mother only received thyroid *before* mating. She required over four months to recover from the thyroid influence.

These eight cases reported here as well as numerous others of which the detailed history cannot be given leave no doubt that the

excess of thyroid in the system greatly interferes with the breeding qualities of the animals. Twenty-four matings in which both the parents were treated resulted in failure, 2 in which the male alone had been treated and 4 in which the female alone received thyroid food, all in all 30 matings. Yet out of these 14 males, 7 had been tested and given offspring previously to the treatment, and out of the 16 females 9 had been tested and found fertile.

In a number of cases the female died before pregnancy began or before a live litter was born. The length of time these females were kept with the males is given in the following table:

1. ♀ dies after 2 days		Thyroid given after mating.
2. ♀ dies after 2 days		
3. ♀ dies after 7 days	no pregnancy	
4. ♀ dies after 19 days		
5. ♀ dies after 21 days		
6. ♀ dies after 21 days		
7. ♀ dies after 38 days		
8. ♀ dies after 57 days 6 fetuses		No thyroid given after mating.
9. ♀ dies after 67 days 7 fetuses		
10. ♀ dies after 107 days pregnant		
11. ♀ dies after 112 days pregnant		

The continuation of this table gives the time that elapsed between mating and birth of live litter:

12. Young born after 24 days ♀ *normal* All die early	No thyroid given after mating.
13. Young born after 26 days ♀ *normal* All die within two weeks	
14. Young born after 27 days ♂ fed on normal food full month previous to mating ♀ fed on thyroid 2 months only	
15. Young born after 28 days ♀ *normal* 2 die within a week	
16. Young born after 110 days 87 days after thyroid treatment	Thyroid feeding continued for 33 days after mating.

17. Young born after 113 days ♂ *normal* 18. Young born after 117 days ♀ *normal* 19. Young born after 151 days ♂ *normal*	No thyroid given after mating.

This enumeration of 19 matings shows that pregnancy did not set in until several weeks after the discontinuation of the thyroid feeding. Whether or not copulation without fertilization took place before that time, cannot be stated. Surely attempts to copulate were made by the several males.

In those cases in which the litter was born within less than 30 days after mating,[3] the mother was normal, viz., not fed on thyroid (Nos. 12, 13, 15). Still the young died soon after birth. In the one case in which both parents had been treated and yet pregnancy set in early (No. 14), the male had been fed on normal food one month previous to the mating, while the female had been treated with thyroid for two months only. In cases 17–19 one of the two parents was normal, but it took the treated partner over three months to recover from the thyroid influence.

Thus under no circumstances will the continuation of the thyroid treatment allow pregnancy to set in. Numerous matings of this kind resulted in failure. Also in case 16 fertilization did not occur until 63–65 days after the thyroid feeding was stopped.

The feeding to rats of fresh thyroid tissue shows its effect in three different ways:

1. When the dose is too large, all the well known symptoms of hyperthyroidization become evident, viz., emaciation, diarrhoea, muscular weakness and finally cachexia leading to death. The hair becomes yellowish, stands erect, sometimes falls out in patches, in short the entire coat looks ragged.

2. When the dose is so regulated as to keep the animals in apparently good health—the fur will always become shabby—then the animals do not breed. Not one mating of both parents treated gave any result, when the feeding continued after the animals had been placed together. Pregnancy was always

[3] The gestation period of the rat is from 21–23 days.

delayed, since fertilization did not occur until several weeks after the application of the thyroid had been discontinued.

3. Did pregnancy finally occur, it resulted

(a) In abortus,

(b) The young died soon after birth,

(c) In very late pregnancies, the young show a diminished tendency to grow. Although they are not especially frail, they keep in relative size behind the normally fed rats of the corresponding age.[4]

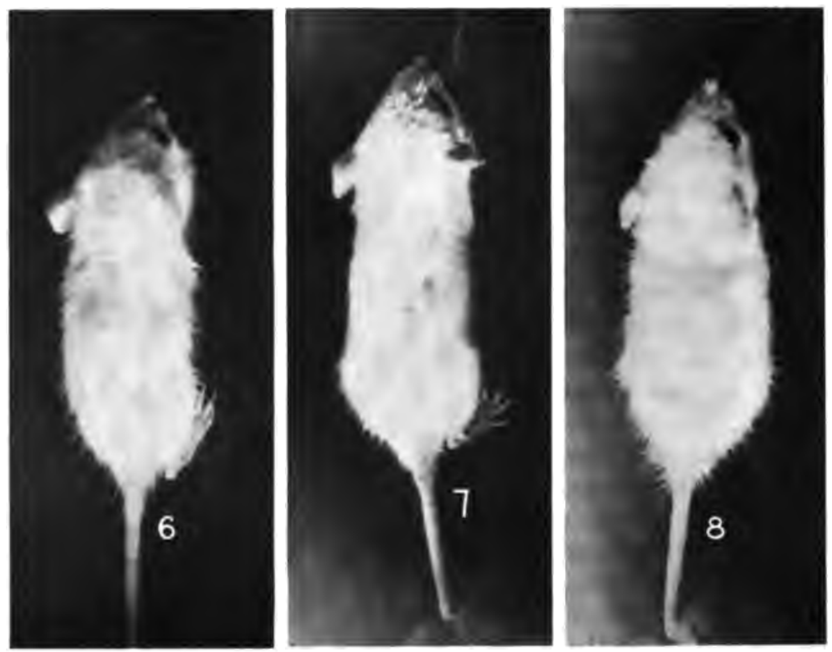
The question now presents itself, whether the symptoms enumerated under (2) and (3) are simply the consequences of the general weakening effect of the thyroid food (1), or whether the genital glands have been affected so as to injure the sex cells. From the various reports in the literature on the interaction of thyroid and genital glands, especially under pathological conditions, this question is justified.

It may at once be said that the delay of pregnancy (2) is in all probability due to the weakening effect of hyperthyroidization. It seems, however, that this cannot fully account for the effects of the thyroid treatment, as stated under (3). It is reasonable to hold the latter responsible for the death of the several mothers during pregnancy and the reduced vitality of the offspring of early pregnancies. But the slackened growth of the young of late pregnancies can hardly be solely due to the weak condition of the mother. It can surely not be due to that condition in those matings in which the father only had been treated, while the mother was a normal female. It seems rather, although at present this is a mere assumption, as if the tendency to grow, inherited from the two parents, had been checked in some way. Former experiments already reported and some under way clearly show that the rate of growth and differentiation of a generation can be influenced by the application of thyroid, but there

[4] There is considerable variation in size and weight between the several stocks of white rats raised in different laboratories. For information on this point I am much obliged to Professor H. H. Donaldson, of the Wistar Institute. I also refer to his various papers on this question. The thyroid treated rats do not in a given time reach the length and weight of our normal rats. Compare illustrations.

with thyroid.

Normal rat, 34 days old.



Figs. 6-8. One-third reduction. 3 rats 38 days old. Mother treated with thyroid.

9
Fig. 9. One-third reduction. Normal rat, 34 days old.

is no evidence yet that this influence can be transmitted to the second generation. If it could be shown that the rats of the second generation, although stagnant in growth, differentiate and mature at the same rate as or faster than normal rats, the proof for the correctness of the above assumption could be given.

This preliminary report is supposed to state only some of the actual observations so far made. The histological data concerned will be reported, when the experiments will have been carried sufficiently far.

THE CHANGES IN THE CONTENT OF HAEMOGLOBIN AND RED CORPUSCLES IN THE BLOOD OF MAN AT HIGH ALTITUDES

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That the number of red corpuscles and the content of haemoglobin are increased in the blood of the peripheral capillaries by residence at high altitudes has long been known. In recent years a considerable number of investigators have attempted to determine whether the change is brought about by the mere loss of fluid from the blood without any alteration in the total number of corpuscles or total amount of haemoglobin in the body, or whether the essential thing is an actual new formation of red corpuscles and haemoglobin.

The question at issue has been answered in the affirmative for an active new formation. The problem has been approached in a variety of ways. Thus the observations of Zuntz and coworkers (1) indicated that there was an absolute increase in the amount of haemoglobin and number of red corpuscles, since examinations of stained sections of the bone-marrow from dogs at sea-level and from dogs kept at a high altitude showed for the latter a decrease in fat-cells and an increase in the blood forming elements. This they thought showed an increased activity of the corpuscle producing centers at the high altitudes.

Abderhalden (2) from an extensive series of observations on rabbits and rats, at an altitude of 6,100 feet, concluded that the amount of haemoglobin per animal was not altered, though the amount per kilo body weight as well as the percentage value of the haemoglobin and red corpuscles rose, in that the weight of the animals was uniformly less at the high altitude. He took this to indicate a concentration of the blood without overpro-

duction of haemoglobin. Abderhalden used Welker's method of washing out the blood for the determination of the total haemoglobin and blood volume. Dreyer and Walker (3) have recently gone over Abderhalden's data and found that the blood volume of the rabbits diminished 10.7 per cent at the high altitude, while the haemoglobin readings increased 25.1 per cent. They found that 13.1 per cent of the increase in the haemoglobin was due to a new formation of haemoglobin and that only 12 per cent was due to the diminution in the total blood volume.

Douglas, Haldane, Henderson, and Schneider (4) by the carbon monoxide method of Haldane and Lorrain Smith determined the total amount of haemoglobin and the blood volume of four men during a residence of five weeks on Pike's Peak, altitude 14,109 feet. They found that during the first few days of residence the blood concentrated but that afterwards there was a large increase in the total amount of haemoglobin and a return to, or even a slight increase above, the normal blood volume.

Laquer (5) on Monte Rosa, found that dogs deprived of haemoglobin by hemorrhage of half of their blood supply regenerated it in about 16 days, while at the lower altitudes 27 days were required for the restoration after a similar hemorrhage. These results, like those of Zuntz and co-workers, show the blood forming centers to be more active at high elevations.

Schneider (6) by the carbon monoxide method followed the blood changes in a man who after living six months at an altitude of 14,109 feet returned to a level of 6,000 feet. The total oxygen capacity of the blood decreased gradually in the course of ten weeks 11.9 per cent. This subject destroyed in the period a surplus of 98 grams of haemoglobin. Thus it was shown that there had been an overproduction at the high altitude.

Other recent researches support the evidence that there is an actual increased production of red corpuscles and haemoglobin. Bürker, Jooss, Moll, and Neumann (7) studied the changes in the red corpuscles and the qualitative and quantitative changes in the haemoglobin in four men at Sanatorium Schatzalp, altitude 6100 feet, and found the increase to be an absolute rather than a relative one. Furthermore the blood did not resume its low

altitude composition within a month after they returned to sea-level. Cohnheim and Weber (8) examined the blood of 23 men working on a railway on the Jungfrau Peak in the Alps and concluded that there was no alternative explanation of the increase in corpuscles and haemoglobin save a new formation.

The rise in the number of erythrocytes and the percentage of haemoglobin is especially rapid during the first two to four days of residence at the high altitude, after which there occurs a more gradual increase for about three weeks. Abderhalden's rabbits taken from Basel, 870 feet, to St. Moritz, 6100 feet, showed a percentage increase in haemoglobin of 17.3 the first days. Dreyer and Walker in their review of Abderhalden's data conclude as follows: "But in animals examined within a day or two of their ascent to a high altitude the whole change is due to diminution of the blood volume."

Douglas, Haldane, Henderson, and Schneider are of the opinion that three of their four subjects showed a diminished blood volume during the first days of residence on Pike's Peak. The subject studied most carefully had on the seventh and eighth days about a 15 per cent increase in the haemoglobin and a total blood volume 10.8 per cent less than in Colorado Springs. They concluded that "the increased percentage of haemoglobin on Pike's Peak was apparently due in part, during the first few days, to concentration of the blood."

Evidence that the early increase in erythrocytes and haemoglobin may be due to other factors than the loss of water from the blood is found in a report of experiments by Campbell and Hoagland (9). They carried three rabbits to the summit of Pike's Peak and on arrival counted the number of red corpuscles in the blood taken from the ear and from the mesentery. The average count from the ear in Colorado Springs was 6,087,000 and on Pike's Peak 6,635,000, an average increase of 9 per cent. The average count from the mesentery on Pike's Peak was 5,897,000 or 11 per cent less than the count for the external capillaries at that altitude. They concluded that the early increase in corpuscles was due to a changed vasomotor condition in the peripheral vessels.

BLOOD CHANGES DURING ABDOMINAL MASSAGE

We have recently made two series of observations which shed some light on the rapid increase in haemoglobin and red corpuscles that occurs in the peripheral blood vessels during the first days of residence at a high altitude. In connection with another study (10) in Colorado Springs we had found that massage of the abdomen, or pressure and massaging action obtained by placing on the abdomen a heavy weight of lead foil, weighing about 25 pounds and so shaped as to fit between the lower ribs and the top of the pelvis, invariably increased the amount of haemoglobin and the number of red corpuscles in the peripheral capillaries. This concentration of the blood was caused by the driving onward into the general circulation of a large number of erythrocytes which had been lying dormant in the splanchnic area.

All haemoglobin determinations recorded in this paper were made with the Haldane-Grower haemoglobinometer and the red corpuscles have been counted with the Thoma-Zeiss haemocytometer.

Abdominal massage and pressure experiments on men who had resided some days on Pike's Peak failed to cause the usual increase in the red corpuscles and haemoglobin. Six men served as subjects for the tests. In each experiment the normal content of haemoglobin and number of erythrocytes was first determined in blood taken from a finger. Then with the subject reclining on a bed the abdomen was vigorously kneaded for five or more minutes, after which the weight was placed in such a manner that the respiratory movements continued the massage. In several of the experiments the weight was removed after a time and active massage again given for another period of five minutes. Two determinations of the haemoglobin and red corpuscles were made during each experiment, the first at the end of five or ten minutes and the second after fifteen minutes of massage and pressure. The results appear in Table I. In Colorado Springs a similar treatment of the abdomen raised the haemoglobin content of the blood 3.5 to 7 per cent and increased the number

of red corpuscles 7 to 9 per cent. In only two of the twelve experiments on Pike's Peak was a concentration obtained. In two others a definite change was not evident, while in eight instances the blood was diluted, the content of haemoglobin decreased as much as 1.3 to 3.1 per cent and the number of red corpuscles 0.7 to 5.4 per cent.

The two observations in which concentration occurred find an explanation in the fact that the two men, Atwater and Gregg, had only the day before walked up the Peak and had not at the

TABLE I

Blood changes during massage on Pike's Peak

SUBJECT	DATE	NORMAL		AFTER 15 MINUTES OF MASSAGE			
		Haemoglobin	Erythrocytes	Haemoglobin	Per cent of change	Erythrocytes	Per cent of change
Atwater[1]	June 20	113	6,080,000	118	+4.4	6,560,000	+7.9
	June 23	131	7,197,000	128	−2.3	6,856,000	−4.7
Gregg[1]	June 20	114	6,216,000	122	+7.0	6,776,000	+9.0
	June 22	123	6,744,000	120	−2.6	6,496,000	−3.6
	June 23	121	6,640,000	122	+0.8	6,644,000	+0.1
Havens[2]	June 22	135	7,296,000	132	−2.2	6,904,000	−5.4
	June 26	129		127	−1.6		
Schneider[2]	June 21	120	6,636,000	117	−2.5	6,384,000	−3.8
	June 26	126	6,640,000	124	−1.6	6,592,000	−0.7
Sisco[2]	June 21	132	6,928,000	132	0.0	6,944,000	−0.2
	June 26	126	6,768,000	122	−3.1	6,528,000	−3.5
Robison	June 28	147	7,744,000	145	−1.3	7,504,000	−3.1

[1] Ascended on foot June 19.
[2] Ascended by railway June 16.

time clearly reacted to the influence of the lowered barometric pressure. They arrived at the summit about one o'clock in the afternoon, having climbed four hours. The following morning at eight o'clock Atwater gave a haemoglobin reading of 124 and an erythrocyte count of 6,888,000. This was above his Colorado Springs average of 112 for haemoglobin and 6,224,000 for erythrocytes. Gregg had in Colorado Springs an average of 116 for haemoglobin and 6,514,000 for red corpuscles. Determinations made on him the first morning on Pike's Peak showed practically

no change, haemoglobin 118 and erythrocytes 6,484,000. Neither of the men felt especially well that day and when examined at 4.30 in the afternoon each showed that there had been a decided fall in the haemoglobin and erythrocytes since early morning. It was noted, however, that Atwater's blood was still more concentrated than it had been in Colorado Springs. Gregg's, on the other hand, had fallen below his Colorado Springs average. It was at this time that abdominal massage and pressure caused the marked increase for both men in the content of haemoglobin and erythrocytes in the blood of the peripheral capillaries. Atwater showed as a result of the treatment an increase of 4.4 per cent in haemoglobin and 7.9 in red corpuscles and Gregg had increases of 7 and 9 per cent respectively.

On the following day, June 21, each man made a run, the results of which are given later, so that abdominal massage was not applied again until the 22d. On that day Gregg alone served as subject and then instead of being concentrated the blood was diluted by the massage, the haemoglobin falling 2.6 per cent and the red corpuscles 3.6 per cent. On the 23d Gregg's blood was not as concentrated as on the 22d, hence it is not surprising to find that massage caused a very slight rise in the haemoglobin and red corpuscles. On the 23d Atwater's blood showed the same diluting reaction that massage caused in all the other men we examined on Pike's Peak.

The single observation on Robison, the resident manager of the Summit House, is of more than passing interest in that he had then resided on the summit nearly six weeks. He, too, showed a dilution of 1.3 per cent in haemoglobin and 3.1 per cent in red corpuscles.

These data show that the splanchnic area does not contain in men after the preliminary period of adaptation the large reserve of corpuscles that it holds inactive at the lower altitudes. The diluting effect of massage very likely is caused by forcing lymph from the abdominal viscera into the blood.

THE BLOOD CHANGES DURING PHYSICAL EXERTION

It has been shown by a number of writers (11) that physical exertion at low altitudes causes a pronounced concentration of the blood of the peripheral capillaries. In another paper (10) we have demonstrated that the increase in haemoglobin, in the number of erythrocytes, and in the specific gravity of the blood which occurs during muscular activity results from the sudden passage into the blood of a large number of red corpuscles lying dormant in the body, chiefly in the splanchnic area. In Colorado Springs we have without exception, in the forms of exercise used by us, found the blood to be concentrated during the exertion. The haemoglobin was found to increase 3.5 to 11 per cent and the number of red corpuscles 3.2 to 22 per cent.

On Pike's Peak two forms of exercise similar to those used in Colorado Springs were employed; the first a run on the level of a half and also three quarters of a mile requiring three and a half to five and a half minutes, and second a dash of 175 yards up the "Cog" road track in from thirty-five to forty-five seconds. Six men served as subjects for a total of twelve trials. The results appear in Table II. In eight of these exercise experiments the effort caused no increase in the concentration of the blood, or even resulted in a slight dilution. The first test with Atwater who had walked up was made after he had been on the summit fifty hours. At that time the haemoglobin increased 1.7 per cent, but a similar run two days later when he had reacted well to the altitude failed to concentrate his blood. With Gregg, however, a concentration was obtained after each of the runs made by him. In the first, after a sojourn of fifty hours, the haemoglobin increased 3.5 per cent and the red corpuscles 8.5 per cent. Gregg made his second run forty-eight hours later when he was known to be in better condition. The haemoglobin then increased only 1.6 per cent and the red corpuscles 2.3 per cent. In this connection it is interesting to find that in a sojourn of four days on Pike's Peak Atwater's haemoglobin rose from his Colorado Springs average of 112 to 131, or 17 per cent, and the red corpuscles increased from 6,224,000 to 7,192,000, or 16 per cent. On the other hand

Gregg's haemoglobin on the fourth day had increased from his Colorado Springs average of 116 to 121 only, or 4 per cent, and the erythrocytes had increased from 6,514,000 to 6,640,000, or 1.9 per cent.

The above observations suggest that as a person reacts to the influence of the high altitude the reserve corpuscles are thrown into the general circulation. As a consequence there is in individuals who have reacted well to the lowered barometric pressure no reserve to bring out during strenuous physical exertion and for

TABLE II

Blood changes during exercise on Pike's Peak

PERSON	DATE	NORMAL		AFTER THE RUN	
		Haemoglobin	Erythrocytes	Haemoglobin	Erythrocytes
		Ran from 0.5 to 0.75 mile			
Atwater...	June 21, '14	118	6,392,000	120	6,432,000
	June 23, '14	133	6,984,000	133	6,912,000
Gregg.....	June 21, '14	115	6,100,000	119	6,616,000
	June 23, '14	122	6,640,000	124	6,792,000
Havens....	June 2, '13	126	6,900,000	126	6,900,000
	June 27, '14	129	7,048,000	130	7,104,000
Sisco......	June 1, '13	120	6,900,000	120	6,800,000
	June 27, '14	128	6,736,000	129	6,712,000
		175 yards up the "Cog" track			
Eager.....	Oct. 26, '13	115	6,472,000	114	6,238,000
Havens....	June 28, '14	129		126	
Robison...	Oct. 27, '13	150	8,256,000	152	8,376,000
Sisco......	June 28, '13	134		133	

this reason the blood does not concentrate during exercise at the high altitude as it regularly does at lower altitudes.

It is unfortunate that we did not have Gregg run on the 22d when it was found that abdominal massage diluted the blood. On the 23d an abdominal massage experiment made two hours before the run raised the haemoglobin 0.8 per cent and slightly increased the number of red corpuscles. The fact that such strenuous exertion as a half mile run increased the haemoglobin only 1.6 per cent makes it probable that longer continued and more vigorous massage would, very likely, have raised the haemo-

globin content an equal amount. We believe that these experiments indicate that the splanchnic area is the chief reserve of red corpuscles.

Robison, the resident manager of the Summit House, kindly ran for us a distance of 175 yards up the steep grade of the "Cog" road on October 27, 1913. He had then resided on the summit of the Peak over five and a half months. This run increased his haemoglobin 1.3 per cent and the red corpuscles 1.4 per cent. The same autumn ten days after he had descended to Colorado Springs he ran again in one of the college buildings doing about the same amount of work in stair climbing. His haemoglobin then increased from 142 to 149, or 4.9 per cent, and the erythrocytes from 6.8 to 7.4 millions, or 8.8 per cent. Our abdominal massage experiment was made on him the following summer when he had been on the summit less than six weeks. It was impossible to try a work experiment at that time. These observations on Robison suggest that even in those acclimatized to very high altitudes there is not a large reserve supply of corpuscles available to meet the increased demand for oxygen during exertion.

Laquer (5) reported that after hard muscular work there was a decrease in the concentration of the blood of the fingers at the high altitude, this he concluded was the result of a vasomotor change. We found on Pike's Peak that the content of haemoglobin and erythrocytes immediately at the end of exercise was usually the same as before exercise. Later, however, the blood slowly diluted just as it does at lower altitudes. Hence within a few minutes the concentration will show a decrease. Often this diluting process was more marked than in Colorado Springs.

Our two series of observations on the influence of abdominal massage and pressure, and of muscular exertion on the blood changes show that a part of the rise in the number of erythrocytes and in the percentage of haemoglobin that occurs during the first two to four days of residence at high altitudes is caused by throwing into the general circulation a large number of corpuscles that have been inactive in the splanchnic area, and very likely to some extent elsewhere, at lower altitudes. The stimulus that calls forth these resting cells is quite likely associated with the

demand of the tissues for oxygen. At high altitudes a need for oxygen is felt even during rest. That there is a shortage of oxygen during the first days of residence has been clearly shown by Douglas, Haldane, Henderson, and Schneider.(4). All available corpuscles are soon thrown into the general circulation in the effort to supply this need, with the result that during muscular work there are no corpuscles remaining in reserve, consequently there is no increase with exercise.

The changes in the blood on adaptation to high altitudes may here be briefly summarized. A rapid increase in the number of red corpuscles and percentage of haemoglobin in the blood of the peripheral vessels occurs during the first two to four days of residence at the high altitude, then follows a more gradual increase for about three weeks. The initial rapid increase is brought about in part by throwing into the systemic circulation a large number of red corpuscles that under ordinary circumstances at low altitudes are side-tracked and inactive; and in part by a concentration resulting from a loss of fluid from the blood. The more gradual increase in red corpuscles and haemoglobin extending over several weeks is brought about by the increased activity of the blood forming centers so that there results a large increase in the total number of corpuscles and amount of haemoglobin.

THE SEQUENCE OF BLOOD CHANGES DURING THE EARLY DAYS OF RESIDENCE ON PIKE'S PEAK

The rise in the haemoglobin content of the blood has been followed in three subjects during three to five expeditions to the summit of Pike's Peak. In these several expeditions the rate of increase of the haemoglobin has varied. We believe the differences observed find an explanation in the condition of each subject at the time of the journey. Five men were induced to walk up the mountain so that it was possible to determine the effects of fatigue when added to the influence of lowered barometric pressure. Among our subjects there was opportunity to compare the influence of altitude on men in excellent physical condition because of regular training in muscular activity and on men who

had led an inactive or sedentary life. In three of our expeditions only the change in the haemoglobin content of the blood was recorded while in the fourth the number of red corpuscles was also counted. These data are given in Tables III, IV and V.

TABLE III

(*Haemoglobin*)

DATE	TIME	HAVENS	SCHNEIDER	SISCO	
Average		110	110		Colo. Springs
Oct. 11, 1912	12 noon	110	110		Pike's Peak, just arrived
Oct. 12	10 a.m.	118	117		
Oct. 13	8.30 a.m.	127	124		
Oct. 14	9.30 a.m.	129	118		
Average		116	109	113	Colo. Springs
May 29, 1913	7 p.m.	116	109	112	Pike's Peak 7 hrs. after arrival
May 30	6 a.m.	124	110	118	
May 31	7 a.m.	128	113	121	
June 1	7 a.m.	130	114	123	
June 2	7 a.m.	129	115	121	
June 3	7 a.m.	134			
Average		111	110	111	Colo. Springs
Oct. 24, 1913	7.30 a.m.	113	113	116	Pike's Peak,[1] 1st morning
Oct. 25	7.30 a.m.	114	112	115	
Oct. 26	8 a.m.	115	115	115	
Oct. 27	7 a.m.	119	116	118	
Average		109	109	113	Colo. Springs
June 17, 1914	7.30 a.m.	123	116	120	Pike's Peak, 1st morning
June 18	8 a.m.	126	115	125	
June 19	7 a.m.	129	122	126	
June 20	8 a.m.	130	121	122	
June 21	8.30 a.m.	132	123	130	
June 22	8.30 a.m.	135	121	133	
June 24	8 a.m.	135	126	134	
June 26	8.30 a.m.	134	127	131	
June 28	7 a.m.	129	129	135	
June 29	8 a.m.	132	129		

[1] Havens walked up the Peak October 23, 1913.

All of the subjects of the first two expeditions ascended the mountain in the "Cog" railway train. The haemoglobin content of their blood was determined within a few hours after arrival

and in each subject it was found that the haemoglobin was not altered immediately by entrance into rarefied air. However, in all subjects who had reacted well to the influence of oxygen want a well defined increase in haemoglobin was found to have occurred by the morning after that of the ascent.

Havens has been under observation during four trips to the summit of Pike's Peak (see Table III). Three times the ascent was made by railway car and the fourth he walked up the mountain. Following the three times of passive ascent the increase of haemoglobin was rapid: thus the morning after the ascent in October 1912 it was up eight points by the Haldane-Gower haemoglobinometer, or 7.3 per cent; in May 1913 also eight points, or 6.9 per cent; and in June 1914 fourteen points, or 12.8 per cent. Before each of these three trips Havens had been muscularly active. Prior to the first, in 1912, he had worked out-of-doors all summer at manual labor. In May 1913 he was in perfect training, having exercised daily for three months in preparation for the two mile run in intercollegiate contests. It is interesting to find then that his average for haemoglobin in Colorado Springs was 116 or six points higher than at the time of the first expedition and yet beginning at this higher level the haemoglobin had increased an equal number of points by the first morning after the ascent. In the spring of 1914 Havens again trained for the two mile run for a time but discontinued regular running about three and a half weeks before the trip to Pike's Peak. His normal haemoglobin content dropped during this interval from about 115 to an average of 109. It is interesting to find, therefore, that in this expedition the increase in haemoglobin on the first morning was greater than in the earlier expeditions, having risen from the average lower level of 109 to 123 in about twenty hours. This increase is equal to the sum of the increases obtained the year before by athletic training and altitude influence. In each of these expeditions his haemoglobin reached practically the same level by the third morning of residence on the Peak; these were 129, 130, and 129 respectively. During our residence of two weeks on the Peak in 1914, the haemoglobin reached its maximum—135—in six days. In 1913 it was 134 on the fifth morning.

In the autumn of 1913 Havens made the ascent on foot. While he was not in such splendid physical condition as the June prior he was nevertheless physically strong. However, apparently as a result of the fatigue of the climb, his haemoglobin had increased only 1.8 per cent the morning after the ascent. Furthermore, the rate of increase was less on the following days than it was in the other expeditions. Thus on the third morning the haemoglobin content was 115 instead of 129, the average reading for that day on the other expeditions. On the fourth morning it had risen only 7 per cent above the Colorado Springs average instead of 12 to 19 per cent as it had during the other trips.

Five series of observations on Schneider are available, the four recorded here and a series with the English-American Pike's Peak expedition (4). It is interesting to find that in three of these sojourns Schneider was quite ill with mountain sickness and that during the other two he was not greatly disturbed by the altitude. In the English-American expedition his haemoglobin was up only two per cent the first morning. It increased in the same time in October 1912, 6.4 per cent, May 1913 only 0.9 per cent, October 1913, 2.7 per cent, and in June 1914,. 6.4 per cent. The small rises noted were associated with the three periods of rather severe mountain sickness. Prior to the trip in October 1912 when the haemoglobin increased rapidly Schneider had spent the summer in out-door activities involving considerable physical exertion. Before the trip in June, 1914, at which time the haemoglobin also rose quickly, he had taken regular tramps in the mountains and played tennis several times a week. Before the other excursions Schneider had not taken regular exercise. It appears then that the rate of change in the haemoglobin during the first twenty-four hours spent at high altitudes depends upon the physical condition of the subject.

The rate of change for Schneider also varied in a similar manner on the subsequent days. Thus for the two trips when he was in excellent physical condition the haemoglobin increased to 124 in two days in October 1912 and to 122 in three days in June 1914. At the time of the English-American Pike's Peak expedition he had just returned from sea-level and his haemoglobin in Colorado

Springs was only 101. Starting from this low level the haemoglobin increased slowly to 122 during the first fourteen days spent on Pike's Peak. The maximum for this trip was 123. In June 1914 the maximum of 129 was reached in twelve days. The maximum obtained in each of the remaining expeditions in four days was only 115 and 116 respectively. These figures are far below those obtained in the expeditions when he was physically strong.

Sisco participated in three expeditions. He never had taken regular exercise, he may be regarded as representing the semi-sedentary type of subject. His haemoglobin increased during the first twenty hours of residence on the summit 4.4, 4.5, and 6.2 per cent respectively in the three sojourns. Sisco had at the time of the ascent in October 1913 a slight bronchitis and suffered some from headaches which may account for the fact that his haemoglobin increased less rapidly this stay than during the other two. During his first trip, June 1913, the haemoglobin increased steadily for three days to 123 and in June 1914 it reached 126 in the same time. In October 1913, however, when he was not well, on the third morning it was no higher than the first morning when it was 116.

In addition to Havens we have had four more subjects ascend Pike's Peak on foot (see Table IV). Eager and Munro walked up with Havens in October 1913. These men were chosen because it was thought that they represented three conditions of physical fitness. Havens, although not in as splendid condition as the June preceding, had exercised the most; Munro and Eager had exercised very little, but Munro seemed the more fit of the two. It is interesting, therefore, to find on the first morning after the ascent that Havens' haemoglobin was up 1.8 per cent, that Eager's had not changed, while Munro's was 2.7 per cent below his Colorado Springs average. In four days Havens' haemoglobin increased 7.2 per cent, Munro's 1.8 per cent, and Eager's had not clearly altered. Havens felt very well throughout the stay on the summit, Eager and Munro could not sleep the first night and suffered more or less with headache throughout their stay.

The morning of June 19, 1914 Atwater and Gregg walked up the Peak and remained on the summit until the evening of the 23d. Atwater reacted well, the morning following the ascent his haemoglobin and red corpuscles were each up 10.7 per cent. That day a headache developed and by afternoon his haemoglobin had decreased from 124 to 113, the red corpuscles from 6,888,000 to 6,080,000. By the next morning both elements had again made a material gain but had not reached the level of the first morning. On the fourth and last morning Atwater's haemoglobin was 17 per cent above his Colorado Springs average and the red corpuscles up 15.5 per cent. He reacted to the influence of the high altitude even better than Havens had after

TABLE IV

Haemoglobin of men who walked up the Peak

MORNING	ATWATER[2]	GREGG[2]	EAGER[1]	MUNRO[1]	
Average	112	116	112	113	Colo. Springs
1	124	118	112	110	Pike's Peak
2	120	117	114	113	
3	122	123	115	114	
4	131	121	113	115	

[1] Walked up October 23, 1913.
[2] Walked up June 19, 1914.

his climb in the October trip. Atwater had for ten or more days prior to his trip been working regularly out-of-doors as a house painter. It was his opinion that he was in most excellent physical condition.

The morning after the ascent Gregg showed an increase of only 1.7 per cent in haemoglobin and no definite change in the number of red corpuscles. He suffered from mountain sickness the first night on the Peak and had a headache the first two days. Gregg had not followed a system of regular exercise and had led only a fairly active life before joining the expedition. In four days his haemoglobin increased only 4.3 per cent and the red corpuscles only 1.9 per cent. His increase in corpuscles throughout the stay was uniformly less than that of the haemoglobin.

The red corpuscles were counted only in the expedition of June 1914. It will be observed, however, that they and the haemoglobin, in all subjects except Gregg, varied together and about equally. These counts appear in Table V.

The data obtained in our study of the changes in the haemoglobin and red corpuscles in these several expeditions show that both, in physically fit persons who ascend the mountain passively, increase rapidly within twenty-four hours. This reaction does not begin immediately on entrance into the rarefied air. There

TABLE V

(*Red corpuscles per cubic millimetre*)

DATE	HAVENS	SCHNEIDER	SISCO	ATWATER*	GREGG	
Average......	6,024,000	5,992,000	6,372,000	6,224,000	6,514,000	Colo. Springs
June 17, '14........	6,872,000	6,472,000	6,732,000			Pike's Peak, 1st morning
18............	7,024,000	6,400,000	6,880,000			
19............	7,160,000	6,800,000	6,720,000			
20............	7,292,000	6,848,000	6,624,000	6,888,000	6,484,000	
21............	7,200,000	6,736,000	6,928,000	6,512,000	6,304,000	
22............	7,296,000	6,472,000	7,032,000	6,656,000	6,744,000	
23............				7,192,000	6,640,000	
24............	7,248,000	6,616,000	7,104,000			
26............	7,000,000	6,656,000	6,856,000			
28............	6,840,000	6,896,000	7,120,000			
29............	6,976,000	6,960,000				

then follows a period of several days, two or three, in which the concentration although somewhat reduced is still quite rapid, after this there may be for a time a continued slow increase. In subjects known to be in poorer physical condition these blood changes occur more gradually and in such subjects symptoms of mountain sickness may appear.

Among subjects who ascend to the high altitude on foot the value of physical fitness or training is very evident. Men in excellent condition react quickly but not, as observations on Havens indicate, so decidedly as when they ascend passively by train. Men not accustomed to strenuous exertion may not react at all the first twenty-four hours after the ascent and only slowly thereafter. We believe one value of physical training is

to be found in the ability of the organism to throw into the general circulation when there is an increased demand for oxygen a large number of red corpuscles that have been side tracked, so to speak, and, therefore, inactive. This throwing of corpuscles into the general circulation accounts in large measure for the first rapid rise in haemoglobin and erythrocytes.

It is the opinion of Dreyer and Walker (3) that the change of blood volume which occurs with a particular change of barometric pressure is proportional to the area of the body surface of the individual. They also believe that the whole increase in haemoglobin and red corpuscles observed within the first day or two after ascent to the high altitude is due to a diminution of the blood volume. If their reasoning is correct the concentration of the blood for a given subject should be about the same each time he goes to a particular altitude. We have shown, however, that in five journeys to the summit of Pike's Peak the haemoglobin content of Schneider's blood had increased in twenty hours as little as 0.9 per cent and as much as 6.4 per cent. Havens and Sisco who also have been with several expeditions have shown similar variations. Dreyer and Walker's explanation fails to account for these variations, while they find an explanation in the theory advanced above.

SUMMARY

1. At low altitudes abdominal massage increases the number of red corpuscles and percentage of haemoglobin in the peripheral capillaries. In men partially or wholly acclimatized to a high altitude abdominal massage lowers the content of haemoglobin and red corpuscles. Before the subject reacts to the influence of lowered barometric pressure, abdominal massage may raise the content of haemoglobin and red corpuscles.

2. Physical exertion at low altitudes concentrates the blood but during a sojourn of four days to two weeks at a high altitude this reaction occurs only in the period before the number of red corpuscles and percentages of haemoglobin have increased. In a subject who had lived five and a half months at 14,109 feet a given exercise caused a slight concentration of the blood but not as much as it did at the altitude of Colorado Springs.

3. While there is a reserve supply of corpuscles at low altitudes this is lacking for some time during residence at the high altitude.

4. The number of red corpuscles and percentage of haemoglobin do not increase immediately on arrival at the high altitude. Usually there occurs within twenty-four hours a marked increase in both.

5. The rise in haemoglobin and red corpuscles for a particular subject during the first three or four days spent at a high altitude is not the same for different visits. The increase is most rapid in subjects who have taken regular exercise before ascending to the high altitude.

6. Fatigue due to walking up a mountain delays the altitude increase in haemoglobin and red corpuscles.

7. The rapid increase in the number of red corpuscles and percentage of haemoglobin the first two or three days spent at a high altitude is due in part to the throwing into the general circulation of a large mass of reserve corpuscles, and in part to a loss of fluid from the blood. The blood forming centers also become more active and increase the total number of corpuscles and total amount of haemoglobin.

We wish here to express our hearty appreciation of the twelve Colorado Springs friends who generously supported our expedition in June 1914, and to the young men who so kindly served as subjects.

BIBLIOGRAPHY

(1) Zuntz, Loewy, Müller, and Caspari: Hohenklima und Bergwanderungen. 1905, p. 172.
(2) Abderhalden: Zeitschrift f. Biologie, 1902, xliii, p. 125.
(3) Dreyer and Walker: Lancet, 1913, clxxxv, p. 1175.
(4) Douglas, Haldane, Henderson, and Schneider: Philosophical Transactions, Royal Society of London, 1913, Series B, ciii, p. 271.
(5) Laquer: Deutsches Archiv f. klin. Med., 1913, cx, p. 189.
(6) Schneider: This journal, 1913, xxxii, p. 299.
(7) Bürker, Jooss, Moll, and Neumann: Zeitschrift f. Biologie, 1913, lxi, p. 379.
(8) Cohnheim and Weber: Deutsches Archiv f. klin. Med., 1913, cx, p. 225.
(9) Campbell and Hoagland: Amer. Jour. of Med. Sc., 1901, cxxii, p. 654.
(10) Schneider and Havens: This journal, 1915, xxxvi, p. 239.
(11) Hawk: This journal, 1904, x, p. 384.

THE INFLUENCE OF LIGHT ON REPRODUCTION IN VORTICELLA

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The aim of the work briefly described in the article, has been to ascertain the influence of monochromatic, and different intensities of light on the reproduction of vorticella.

The vorticella were transferred by means of a Barber[1] capillary pipette, held in a modified triple movement pipette holder.

From a culture of the unicellular organisms, that had been kept covered in the dark, for several months, many encysted zooids were isolated, and transferred to a culture media, consisting of an infusion of Timothy hay. The infusion had been filtered, sterilized by boiling, and kept in sterilized sealed tubes until needed.

The encysted forms were under observation in a hanging drop placed in an open end moist chamber.

As soon as the zooids emerged from their cysts, they were removed to a drop of the sterilized infusion placed on a Hemocytometer micrometer slide. By this means, the direct increase in organisms could be ascertained. The slides with the young zooid were placed in a Petri-dish that contained a thin layer of water to prevent evaporation of the solution. The organisms of the same age thus secured, were kept for each series of observations, under the same conditions of temperature, moisture, food and intensity of light.

To ascertain the effect of colored rays, blue, green, yellow and red glass, gelatin films, and reflected rays from Hering's monochromatic colored cards were employed. The observations thus

[1] Barber M. A.: Jour. of Infections Dis., 1911, Vol. viii, p. 348.

secured were compared with those made in sun or electric light of constant intensity; the colors were placed both in bright and dim light and so that the reflected or transmitted rays penetrated the culture either from above or below. The heat rays were cut out by water contained in parallel sided glass dishes placed in the path of the beams of light.

In order to study the influence of the intensity of light, the slides with the vorticella were placed either half or one and a half or two meters from a window having southern exposure or electric light. Some were placed in a large blackened wall box or in a box covered with smoked glass to vary the intensity of the rays of sunlight.

From the observations that were obtained from the various experiments the following general conclusions were deduced.

1. Vorticella exposed to daylight for intervals of four days increase in number more rapidly on bright sunny days than on cloudy dark ones.

2. When the zooids are placed at different distances from the source of light on an average 25 develop one half meter, ten one and one half meter from the light and two in the dark.

3. In comparing the greatest number that developed on sunny days from one zooid in 24 hours it was seen that 40 grew under green, 29 under yellow, 26 under white and 13 under blue, 17 under red.

4. The average rate of increase on cloudy days or under dim light was 4 for yellow, 3 for green, 2 for blue, white or red.

5. We conclude that the stimulating effect on the reproductive power of vorticella increases up to an optimum with increased intensity of light, and that the bright luminous rays of yellow and green are more effective as stimulating agencies on the reproductive power of vorticella than are the red or blue rays.

EXPERIMENTS ON X-RADIATION AS THE CAUSE OF PERMEABILITY CHANGES

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Studies on the effect of radiation by Roentgen rays upon eggs have shown that these rays are capable of causing changes in the activities of the egg and of certain of its parts. Among others, changes in the rate of cell division, differences in the behavior of chromatin in division, and variations in the activity of cell extracts have been noted as the result of exposure to these rays. Some, at least, of the departures from normal are similar in character to the results obtained by applying various experimental methods to living eggs. It has been suggested therefore that changes in the permeability of the plasma membranes of the egg cells may be the basis for the abnormal conditions observed. This suggestion comes from the fact that changes of such nature are held to be responsible for cytolysis of the cortical layer of the egg substance, and the processes which, as initiation of cell division according to Loeb, are consequent to cytolysis; for metabolic changes such as the increase in the elimination of CO_2 and of catalase and increase in oxygen absorption; and for various other physiological reactions of the cell.

There would seem to be a number of a priori reasons for expecting permeability changes to be brought about by radiation. McClendon found evidence for the increase in permeability of the sea urchin's egg at the beginning of development, and he states that "there is some indirect evidence that increase in permeability may cause an increased division rate in tissue cells. Though cell growth may influence division, it is probable that permeability influences growth." My observations that expos-

sure of Planorbis eggs to X-rays hastens the rate of division, could be most easily explained, perhaps, on the basis of this assumption. McClendon also cites the case of cancers that have been produced by the action of X-rays upon the skin. "The cells in the skin were changed so that they proliferate more rapidly. Similarly, electrical changes have been observed to start the egg cell to rapid proliferation" (tissue cells have also been electrically stimulated to increased division). He thinks that "there is some irreversible change in the permeability of these cells." It is, furthermore, to be expected that perhaps an agent which brings about the profound internal disturbances known to be caused in the chromatin by radiation, as shown by the researches of the Hertwigs and others, would work extensive changes at the surface where it first comes into relation with the cell, namely, at the plasma membrane.

If permeability changes are caused by radiation, then the general results of that treatment are probably to be brought in line with effects produced by other experimental methods. On the other hand, if no permeability changes are demonstrable it is necessary to look more deeply for the causes underlying the disturbances and it becomes increasingly probable that the effects of radiation are specific, or at least dependent upon some more fundamental principle for their explanation.

Thus the investigation of the question, whether changes in the permeability of the plasma membrane can be brought about by X-radiation, becomes a matter of some theoretical importance. The conclusion reached here rests solely upon the evidence available from experiment.

Four methods for investigating this question were available to the writer. (1) All agents which cause permeability changes cause an exudation of pigment from the integument of Arenicola larvae. (2) The prevailing hypothesis is that the initiation of all division which is the first step in artificial parthenogenesis is due to changes in the permeability of the plasma membrane to cytolysing agents. (3) The indicator method depends upon the sensitivity of neutral red, the indicator, for detecting the presence of alkali, in this case, NaOH, in the cell. (4, an adaptation

of 3). Permeability changes are indicated in certain plant cells where, by the use of an indicator (neutral red in Elodea as suggested by Harvey, '10), the change in content of the cell can be detected when placed in a solution to which the membrane is permeable.

Previous experiences with the physiological applications of X-rays had shown that the effective strengths under the conditions which obtained with my experiments were a short accelerative radiation of two or three minutes on the average, a non-effective one of about five or six minutes, and a longer inhibitive radiation. I have not been able to observe much difference in effect between ten, fifteen and thirty minute radiations, so for most of the work only second of these stronger radiations was used. Apparatus and current were not available for exposures of great intensity or longer duration.

Arenicola larvae. R. S. Lillie has pointed out the usefulness of Arenicola larvae for indicating permeability changes. When these small trochophores "are suddenly brought from sea water into pure isotonic solutions of sodium salts the muscles contract with extreme vigor and persistence, causing the larvae to shorten to half their normal length; at the same time the yellow pigment contained in the cells of the organism diffuse into the solution and colors the latter yellow. The exit of the pigment is the impression of a rapid permeability-increasing or cytolytic action." I have radiated larvae of Arenicola for varying lengths of time (two, five and fifteen minutes at a distance of about four inches from the tube) and have observed them immediately after the radiation as well as at intervals up to an hour following it. No muscular contractions were caused by the radiations. (Increase in permeability here is usually associated with acceleration of movement.) The larvae were injured in no way so far as could be observed; and no pigment diffused out into the water even on the light side of the drop where the larvae were most densely massed due to their strong phototropism. These experiments were repeated and in no case were results obtained different from that just stated. The general conclusion is to be drawn therefore, that radiation does not cause changes in the permeability of these larvae, or that any changes so caused are too slight to be detected by this method.

Artificial parthenogenesis. On the assumption that a substance must enter the egg in order to affect it (that is, the permeability of the plasma membrane must be changed) Loeb has suggested as a test of permeability change the effectiveness of a substance in solution in causing artificial parthenogenesis. Artificial parthenogenesis is held by him to be due to the formation of the fertilization membrane which in its turn is caused by cytolysis of the cortical layer of the egg protoplasm. Cytolysis would imply, therefore, a change in permeability for its occurrence. If not positively proven, this theory without doubt serves excellently as a working hypothesis and it is allowable to proceed on that assumption. Experiments looking toward the production of artificial parthenogenesis by radiation are, therefore, of interest in this connection.

Bohn reported that the eggs of the sea urchin were more easily fecundated after exposure to radium rays although the sperms were rapidly killed. He also records having obtained parthenogenesis. "Si enfin on expose des oeufs non fécondés aux rayons du radium, certains de ces oeufs (2 à 4 pour 100) évoluent sans le concours d'un spermatozoide (parthenogénèse); on obtient des embryons irréguliers, en general des demimorula de 4 ou 8 cellules attachées à un gros blastomère subissant rarement une bipartition." The small percentage of embryos obtained and the slight extent of their development render these results of doubtful significance. The percentage of parthenogenetic embryos of sea urchins now artificially obtained by other agencies are so large that this case should probably be rejected at least until it has been verified by new experiments.

The incomplete parthenogenesis described by the Hertwigs as the result of radiation by radium does not come into consideration here, for by parthenogenesis they mean only the development of the embryo without the participation of the sperm nucleus. The sperm actually entered the egg but was prevented from taking part in subsequent development by the radiation. But it was able to initiate development before this destructive effect occurred, and it is the initiation of development with which we are here concerned.

The writer performed a number of experiments to see whether X-radiation could be used as a means of inducing development by artificial parthenogenesis. Loeb's "improved method of artificial parthenogenesis for sea urchins (Artificial Parthenogenesis and Fertilization, p. 71) consists of two parts. The first is the treatment with the parthenogenetic agent, e.g., 50 cc. sea water + 2 cc. $\frac{N}{10}$ butyric acid for one and one-half to three minutes; the eggs are then transferred to sea water for about a quarter of an hour. Then the "corrective," the second part of the treatment was given by transferring the eggs to hypertonic sea water (50 cc. sea water + 8 cc. $2\frac{1}{2}$ M NaCl) for twenty to twenty-five minutes. There are, of course, many other methods of causing artificial parthenogenesis, but as this method is well worked out and has become a standard, which is almost certain to give results, only it and modifications of it were tried for the experiments here described. Perhaps other methods might have given results of different character but for various reasons it was not thought feasible to try them. Attempts were made to use radiation in place of both the first and second treatments just described and also in connection with certain parthenogenetic agents to accelerate or retard them actively. The following experiments are selected from the entire number performed as examples.

EXPERIMENTS UPON NEREIS EGGS

July 23, 3.15 p.m. Radiated Nereis eggs two minutes (Labelled N. 2), kept part of N. 2 for control. It was not further treated. No jelly nor polar body had been formed by 10 p.m. next day.

3.34. Put second part, N. 2. a, in sperm suspension (fertilized with sperm normally).

4.50. N.2.a shows beginning of cleavage furrow.

5.05. Cleavage furrow nearly complete.

6.00. Second cleavage.

July 24. 10 a.m. .95 per cent normal ciliated embryos.

July 23 3.34. Put third part, N.2.b, in KCl $\frac{M}{4}$ for twenty minutes. Then divided it into two lots.

3.54. One lot, N. 2. b sp., was fertilized with sperm suspension.

4.38. N. 2. b sp. showed first polar body.

July 24. 10 a.m. Cytoplasmic masses somewhat irregular in character. No development.

July 23. 3.54. Second lot of N. 2. b placed in sea water, labeled N. 2. b. w.

4.38. N. 2. b. w. showed first polar body.

July 24, 10 a.m. Cytoplasmic masses irregular in shape; show "segregation." No parthenogenesis.

July 23. At this same time another series of experiments of the same character were performed upon other eggs of the same lot. These eggs however were radiated fifteen minutes, beginning at 3.15 p.m. (N. 15)

Kept part of N. 15 for control. It was not further treated.

July 24. 10 a.m. No jelly or polar body had been formed.

3.35. Put part, N. 15 a, into sperm suspension, normal fertilization.

4.50. N. 15 a. shows beginning of cleavage furrow.

5.05. Cleavage nearly complete.

6.00. Four-cell stage.

July 24. 10 a.m. 99 per cent normal ciliated embryos.

July 23. 3.35. Put part of N. 15 into KCl $\frac{M}{2}$ for twenty minutes, N. 15 b. These eggs were divided into two lots, one of which (N. 15. b. sp.)

3.55. Was fertilized with sperm suspension.

4.38 N. 15. b. sp. gave off first polar body.

July 24. 10 a.m. Cytoplasmic masses, slightly irregular; they show little segregation, but except for polar bodies give no evidence of development.

3.55. Second lot of N. 15. b. were placed in sea water. N. 15. b. w. No further development except polar body formation, as in N. 15. b. sp.

At the time when these experiments were set up, some normal unradiated eggs were fertilized with unradiated sperm. These (July 24, 10 a.m.) were all alive, were swimming more rapidly, and were in a healthier condition than any of the experiment, even N. 2. a. and N. 15. a. Samples of these as well as of N. 2. a. and N. 15. a. were fixed.

Thus the radiation did not prove effective as a means of starting development in these experiments.

To secure parthenogenetic development in Nereis, the first step is to cause the formation of the egg jelly. There are various methods of accomplishing this, but the one used in a set of experiments to be described here in which jelly formation was brought

about before radiation of the eggs, is to be credited, I believe, to E. E. Just (whose work is as yet unpublished).

The eggs were heated to 35 degrees in water for five minutes. This caused the jelly to form. The eggs were then radiated, part for two minutes and part for fifteen minutes. This treatment ended at 3.31 p.m. At the end of the radiation irregular membranes were to be seen about the eggs. The germinal vesicles did not show. When observed at 3.55 the first polar body had been formed in both and five minutes later the second was given off.

At 6.00 p.m. "segregation" had begun. Some had divided irregularly once and then begun segregation. The same conditions were met with in both radiations.

Some of these eggs had been mixed with sperm after radiation but as the jelly had been formed the sperm were unable to enter and development had not followed. At 6.00 p.m. these, too showed segregation.

The experiments given are typical for Nereis. I was never able to secure results fundamentally different from the above. They point to the conclusion that X-radiation is not an effective means of producing artificial parthenogenesis either in a first or second treatment for Nereis.

[*Note.* In this connection it may be well to call attention to the commonly observed fact that Nereis eggs when first shed are very irregular in shape, owing doubtless to their crowded condition in the animal's body. In the sea water they round up and finally take on the characteristic shape. The phenomena shown here and those concerned with the cortical changes subsequent to fertilization indicate that the membrane is "permeable for both crystalloids and colloids at this time." (Lillie, F. R.) If freshly shed eggs be radiated (whether immediately fertilized or not) and carefully observed in comparison with non-radiated eggs it will be seen that the changes in shape occur at exactly the same time in the two lots. If any change in permeability resulted from the radiation it could scarcely be that both lots should round up at once, or as is the observed fact that the jelly in the fertilized eggs should be extruded in both radiated and

unradiated eggs simultaneously. These observations have been repeatedly made and are in line with the evidence from other experiments that radiation does not cause permeability changes.]

EXPERIMENTS ON ARBACIA

July 29. 9.06. Arbacia eggs were radiated for two minutes. (A 2.)

9.11. Others of same lot were radiated for fifteen minutes. (A 15).

9.33. Both lots were treated with hypertonic sea water (50 parts sea water plus 8 parts 2½ M. NaCl) for twenty minutes and then transferred to sea-water.

10.00. When observed at this time a few eggs in each lot had given off polar bodies.

11.00. A 15. Membranes not formed. But one case of cleavage was observed.

2.30. A 2. Six eggs of the entire lot were observed to have divided irregularly.

A 15. Five eggs of the entire lot had divided.

July 30. A 2. Around the outside of the dish there were perhaps 1½ per cent of swimming blastulae; in the center of the dish where the eggs were somewhat more numerous there were no swimming embryos. Of the whole lot not more than 2 per cent were swimming. Through the dish there were a few fused masses which had lost their pigment but could swim about slowly. Membranes had not been formed on the eggs which did not divide.

A 15. Only one swimming blastula observed in dish which contained quite a large number of eggs. There were no fusions in this dish.

July 31. All had disintegrated.

2 p.m. Some were left standing untreated for a control.

August 2. 3 p.m. About 10 per cent had divided once or twice and disintegrated. No membranes were formed.

This small number of living embryos might be explained as the result of several things. It might be thought that a few sperm in the dish caused the development of these eggs, but the most careful precautions were taken to prevent introduction of sperm and the writer cannot believe that these few eggs were fertilized with sperm. The length of time elapsing before division is what would be expected for parthenogenetically produced larvae rather than after normal fertilization. It is also possible

that these larvae are to be explained, as in the case recorded by Bohn, as the result of radiation. But Loeb has shown that hypertonic sea water itself, without further treatment will cause development in some instances. I have also found a few cases of division in other experiments caused by this treatment alone. In view of this fact and because of the small number occurring in the dishes, it is not justifiable to conclude that radiation will serve as the first factor in causing parthenogenetic development.

Other experiments were performed to find whether radiation could be made to serve as a second factor. On August 1 a series of experiments was set up from the same lot of eggs.

August 1. 2 p.m. Some were treated with hypertonic sea water for twenty minutes and then transferred to sea water.

August 2. 3 p.m. No membranes had been formed. About 5 per cent had divided once or twice irregularly and disintegrated.

August 1. 2 p.m. Other eggs were divided into two lots after having been treated for twenty minutes with hypertonic sea water.

One part (A 2) was radiated for two minutes, and the other (A 15) for fifteen minutes.

August 2. 3 p.m. In both A 2 and A 15 fewer of the eggs had disintegrated than in the other experiments of this set, just mentioned. A few irregular divisions had occurred. No embryos, living or dead, were present in the dishes.

Some of these eggs were radiated two minutes. Half were then treated with the hypertonic sea water. On the next day the following observations were made: half-disintegrated, no membranes, some three-celled eggs, no blastulae.

The other half were radiated but not given any subsequent treatment. No blastula had developed next day and there was a general disintegration of the eggs.

Similarly, some eggs were radiated for fifteen minutes. There were only a few irregular divisions next day and most of the eggs in both parts of this lot (those treated and those not treated with hypertonic sea water) were disintegrated.

It is unnecessary to give further experiments. These will serve to show that the writer was unable to produce artificial parthenogenesis in sea-urchin eggs.

EXPERIMENTS WITH ASTERIAS

It is in general easier to produce artificial parthenogenesis in star fish eggs than in those of the sea-urchin, and Loeb's finding should be noted, that "the starfish eggs differ from those of the sea-urchin in this, that they do not depend upon the second corrective factor with the same degree of necessity." Hence, attention was turned to this form, for if there is any hope of producing artificial parthenogenesis with radiation that result should be obtained here.

Eggs were taken from a starfish and divided into lots, these lots were treated as follows:

1. Eggs were left without treatment of any kind.
2. Eggs were radiated for two minutes, then seventeen minutes later were treated with hypertonic sea water for twenty minutes.
3. Eggs were radiated two minutes. No subsequent treatment.
4. Eggs were radiated fifteen minutes, then treated for twenty minutes with hypertonic sea water.
5. Eggs were radiated fifteen minutes. No subsequent treatment.
6. Eggs were treated twenty minutes with hypertonic sea water. They were not radiated.
7. Eggs were treated as in 6 and then radiated for two minutes.
8. Eggs were treated as in 6 and then radiated for ten minutes.

The observations on these experiments made next day were as follows:

1. Membranes had formed, but there was no further result.
2. Some eggs had irregular membranes. There were no cases of division.
3. Disintegration had taken place. There were no membranes and no signs of division.
4. No evidences of division except a few small cells which looked like isolated blastomeres. One egg had a divided nucleus but showed no cytoplasmic constriction.
5. Disintegration had occurred. There were no membranes and no divisions.
6. Less disintegration was observed than in the preceding case. There were a few small cells.

7. Membranes were formed here; there were a few unsuccessful attempts at division, but none were complete.

8. This dish showed some membranes, some disintegration, but no signs of division.

Eggs from this same female when fertilized with sperm gave over 25 per cent normal swimming embyros, uniformly developed.

Thus no artificial parthenogenesis was obtained with starfish eggs.

It is, of course, impossible to assert that artificial parthenogenesis cannot be brought about using X-radiation as a means until every one of the long series of agents already known with which the radiation might be tried shall have been used; and the present experiments make no claims of being an exhaustive attempt at this end. Rather the experiments here made were modifications of methods ordinarily successful. Yet it seems that if artificial parthenogenesis can be produced in this way, these experiments should have given more indication of it. When viewed in the light of conclusions from other experiments aimed at detecting permeability changes, the failure to produce parthenogenesis by this means seems significant.

On the basis, therefore, of the hypothesis current at this time that the initiation of division with which artificial parthenogenesis starts is due to changes in the permeability of the plasma membrane to cytolysing agents, the experiments to determine whether eggs can be induced to develop in this manner give evidence with their negative results for the conclusion that radiation does not cause any changes of importance in the egg's permeability.

In this connection a late communication by Loeb is of considerable interest. In accordance with his view that cytolytic agents produce artificial parthenogensis, Loeb tried the effect of ultra violet light upon Arbacia eggs. He succeeded in obtaining by this means in some cases fertilization membranes. Without further treatment, the eggs under-went cytolysis without segmentation; if the temperature was lowered, some divided once or twice before cytolysis occurred; "when the eggs were put for twenty minutes into hypertonic sea water, about ten minutes after the treatment with ultra violet light, they developed into

larvae," but scarcely went beyond the gastrula stage. Chaetopterus eggs exposed from five to ten minutes developed into swimming larvae without cell division. He expects Roentgen rays also to cause membrane formation since they too cause cytolysis. I have already noted cytolysis for starfish eggs.

The evidence from my experiments is hardly in agreement with Loeb's interpretation of his observations. It may be that the intensity of the rays which could be produced with my apparatus was not comparable with that of the ultra violet light, or that the two kinds of rays are not physiologically equivalent, but my experiments do not justify the hope that X-rays can be used as a means of producing artificial parthenogenesis. One cannot avoid raising the question, doubtless considered by Loeb himself, whether it was the ultra violet light which actually caused the parthenogenesis, in view of the fact that he has already shown that hypertonic sea water is enough to cause parthenogenetic development in starfish eggs, although he, to be sure, used sea urchin eggs for these experiments.

The indicator method. The indicator method for recognizing permeability changes has been made use of by various investigators but particularly by Harvey who has dicusssed it at length. The test is a more delicate one and the evidence given by it is of more value than that by either of the lines of investigation just discussed. The method involves the staining of the cells, starfish eggs in this case, with an intra vitam stain, which is sensitive to aklali, to act as an indicator, e.g., neutral red. When the concentration of the alkali is great enough or the permeability of the plasma membrane is increased so that the alkali may enter, its presence in the egg is indicated by a change of color from red to yellow.

It is true that when the alkali enters the egg it kills it and for this reason the method has been criticised; it has also been urged that the proteins in the cells as well as other substances present may influence the color change so that it becomes inaccurate and unreliable as a test of the rate of penetration of the dissolved substance into the cell. What the test really shows is the resistance of the cell to the entrance of the alkali. These criticisms

have been considered by Harvey ('13) and he reaches the conclusion as the result of renewed experiment that "the indicator method for the detection of alkali within the cell is therefore a perfectly adequate one." The alkali acts upon the surface of the cell changing the permeability before it enters and kills the cell body. And the difference in the rapidity with which the color change takes place for two alkalies indicates a difference in the permeability of the plasma membrane to the different alkalies.

The method for testing the present problem consists of finding the lowest concentration of the alkali (NaOH) which will just cause the color change. If radiation of the egg will cause increase in permeability, a less concentration of the alkali will then enter and the color change be brought about.

Starfish eggs which had been standing for some time (about 1 hour) were stained with neutral red. The eggs were divided into lots and three of them exposed to X-radiation for two, five and fifteen minutes. They were then tested with a solution of sodium hydrate in magnesium free sea water. (Of $\frac{1}{2}$ M. solutions of NaCl, KCl, and CaCl, 100 parts of NaCl, 2.2 of KCl and 2 of CaCl were taken for the sea water.) The sodium hydrate was diluted to the concentrations of $\frac{N}{100}$, $\frac{N}{200}$, $\frac{N}{400}$, and $\frac{N}{800}$, and the solutions were used to test first the control eggs (stained, but not radiated.) Between $\frac{N}{200}$ and $\frac{N}{400}$ was the concentration which caused the color change. To test the radiated eggs, it was necessary therefore to use only the concentration of $\frac{N}{400}$ and $\frac{N}{800}$. Immediately after radiation the same experiments were made on all of the lots radiated and no difference was detected in their reaction to the test. The same result was observed for all.

The $\frac{N}{400}$ solution did not cause any change in the control, a fresh lot of which was compared with every lot of radiated eggs, and neither this nor the $\frac{N}{800}$ caused a color change which was visible against white paper, but upon examining the experiment under the microscope the observer thought he detected a faint yellow "halo" appearing around the eggs with the first and perhaps to a less degree with both solutions. The reaction, however, was not of decisive character. Later the eggs faded and

died. Increase in permeability cannot be said to be positively indicated by this method, but if the results are to be interpreted as favoring its occurrence, certainly the increase is a slight one.

This test was repeated upon these same eggs about four hours later. No difference in reaction could be observed, indicating that if the radiation caused any increase it was not a permanent one.

The experiment when repeated upon the eggs which had stood four hours gave a similar result, although the yellow "halo" could now be seen about a very few of the control eggs when tested with the solutions of lesser concentration, suggesting perhaps, that the longer interval which elapsed before they were used had slightly lessened their resistance to the penetration of the NaOH.

The experiments and others which were like them on other days gave exactly similar results and established the fact that no marked change in the permeability of the eggs to NaOH followed the radiation, but it left open the question as to whether slight changes might not be caused which would account for the slight yellow "halo." In order to investigate this latter point a new series of experiments was performed exactly as before except that the stained eggs were mixed with a suspension of Chinese ink to bring out the jelly layer and to give a background against which to study refraction effects. The details of the experiment need not be given for the most painstaking observation showed no stain in the jelly and no change in the color of the radiated eggs where exactly the same changes could not be demonstrated in the controls. If the concentration of the NaOH solution was sufficient to penetrate the radiated eggs it was also sufficient to penetrate the control. This observation also led to the opinion that the "halo" was due to some other cause than radiation. No differences existed in the reactions of the two lots of eggs.

These observations all go to show that the radiation causes no change in the resistance of the membrane to the entrance of the alkali, and indicate no increase in permeability.

Elodea cells. The fourth method available for the investigation and the method upon which most confidence is placed

is a direct application of Harvey's experiments on Elodea leaves. This, it will be seen, is in principle the same as the test just preceding. Elodea leaves which are mostly only two cells thick and, therefore, quite transparent, take up neutral red from a solution until they are quite red in appearance. The reasons for choosing Elodea are given in Harvey's paper. As he notes, the stain penetrates the cytoplasm and collects as a red solution in the sap vacuale, indicating a slight acidity of the sap. The cytoplasm and cell walls are usually left unstained. In this condition the leaves form excellent objects for the study of the penetration of various solutions. Many of Harvey's experiments the writer has verified; only that part which bears on the present problem is here discussed.

Harvey found that weak alkalies (NH_4OH and the amines) penetrate very rapidly while the strong (NaOH, KOH, etc.) experience resistance in entering. He further found that the entrance of the strong alkalies is facilitated by the addition of other substances (as chloroform, ether, urea, etc.) in small amounts. The completion of the reaction is shown by the "decolorization," the change from red to yellow of the leaves, and the reaction is a fairly sharp one. Red stained leaves were decolorized on the average in nineteen minutes in $\frac{N}{40}$ KOH + $\frac{M}{8}$ urea in distilled water. If, now, radiation caused change in the permeability of this cell there should be a difference in the times of decolorization of the radiated and non-radiated leaves.

Of all the alkalies tried, a solution of $\frac{N}{40}$ KOH + $\frac{M}{8}$ urea gave the most clearly cut reaction. Red stained leaves were placed in this solution immediately after being radiated for varying lengths of time of from two to fifteen minutes duration. Whole leaves were tested; they were taken from the same or adjoining whorls, for Harvey has shown that widely separated leaves or those from different branches are not comparable. In no case could there be distinguished any difference in the time required for the decolorization of the non-radiated control leaves, the short and the long radiated ones. The average time required, according to my experiments (and this is in agreement with Harvey's data) is nineteen minutes. In a variation of this

experiment the red stained leaves were placed under the X-ray tube while in the alkali solution. Even the constant radiation during the action of the alkali on the leaves did not cause any difference in the time necessary for penetration as indicated by the decolorization of the leaves. Repeated trials of these experiments have given exactly the same results. The rate and penetration of this solution into the Elodea leaves is not changed under the influence of radiation. The conditions are the same where the alkali acts without the added substance. Only a longer time is required for the penetration.

Other of the stronger alkalies act in the same way. It is impossible to distinguish any difference in the rate of penetration into radiated and non-radiated leaves.

Only thirty seconds are required for the penetration of $\frac{N}{40}$ NH_4OH into Elodea cells. Radiated leaves also are decolorized in the same time.

The entire series of experiments on the relative rates of penetration of various alkalies into red stained Elodea cells gave uniformly the same result: the radiation whether shorter or longer does not influence the rate; or, in other words, the radiation brings about no changes in the permeability of the cells for the alkalies under investigation.

Conclusion and discussion. The experiments which have been set forth all warrant the conclusion that the effects which are described by numerous workers as the result of exposure to X-rays are not to be attributed to permeability changes caused by the radiation. Arenicola embryos do not exude pigment under the influence of the radiation. It has not been found possible to induce artificial parthenogenesis with X-rays used either as a first or a second treatment; according to current interpretations artificial parthenogenesis implies changes in permeability. Starfish eggs stained in neutral red are decolorized equally rapidly by sodium hydrate whether or not they have been radiated; and the same thing is true of Elodea leaves and KOH. Evidence from all these lines is in perfect agreement as indicating that no permeability changes are caused by the radiation. The experiments from which the evidence is drawn included only a limited number of substances, it is true, and no

sweeping generalizations may be made from them; but the conclusion is clear that for the substances used no change in the permeability of the plasma membranes is brought about by X-radiation. To assume such changes for other substances is not warranted by the evidence at hand.

Gager's experiments perhaps bear on this problem somewhat. He reports that he obtained only negative results with regard to the effect of radium rays on osmosis, turgidity, and consequent cell enlargement.

While there are certain analogies as mentioned earlier for the causation of permeability changes by radiation, there are at least two facts of common clinical experience which would not lead to that expectation. First, protoplasm is transparent to X-rays; the surface of the cell does not present an obstacle to the passage of the rays, which on the contrary are able to act some distance from the surface of a tissue mass. This, of course, does not preclude the possibility of changes in permeability being induced, but it indicates that the changes are not necessary to the action of the rays. Second, the rays are able to act on cells and tissues which are not bathed by solution. For example, extensive injuries on the skin are caused by exposure to the rays and they clearly do not depend on the entrance of anything to which plasma membranes are permeable into the cells. Neither of these facts precludes the possibility of permeability changes, but both are consistent with the idea that such changes do not occur following radiation.

The trend of physiological investigations in recent years has been away from the idea that particular agencies of experiment cause specific effects on organisms; that is, the same experimental result can often be gotten by various means, as is illustrated by the work on artificial parthenogenesis. On the other hand, some, at least, of the effects of radiation seem of a different character from those of other agencies. Now many of the various means used for causing departures from the normal cycle of events in organisms are known to act by causing permeability changes. It seems not illogical to suggest that the apparent specificity of radiation, in so far as it is real, may be due to the fact that it does not cause such changes, and is not able there-

fore to set up the same series of processes of cytolysis and the like as takes place following action by the more familiar agencies.

Since experimental investigation does not warrant the conclusion that radiation acts by causing changes in the permeability of cells, we must look to other causes for explanation of its effects. The Hertwigs and others have shown that chromatin is injured by the rays and it has also been proven possible to effect the activity of enzymes by radiation. Packard has even suggested that the chromatin injuries are due indirectly to the effect on cell enzymes, and Miss Woodward and the writer have established the capacity of X-rays to modify the activity of the egg extractive, fertilizin. The evidence available at the present time points to a theory of enzyme modification as the best explanation of the effects of radioactivity upon the structure and functions of protoplasm.

REFERENCES

Bohn, G. 1903 Influence des rayons du radium sur les oeufs vierges et fécondés, et sur les premiers stades du dévellopment. C. R. de l'Acad. de Sci., T. 136, p. 1085.

Gager, C. S. 1908 The Effect of Rays of Radium on Plants. Mem. N. Y. Bot. Gardens, iv.

Harvey, E. Newton. 1911 Studies on the Permeability of Cells. Jour. Exp. Zoöl., x.

1913 A Criticism of the Indicator Method of Determining Cell Permeability for Alkalies. Amer. Jour. Physiol., xxxi.

Hertwig, O., G., and P. 1911 Various papers in Arch. f. Mikr. Anat., Bd. 79, et seq.

Lillie, F. R. 1911 Studies in Fertilization I. Jour. Morph., vol. 22.

Lillie, R. S. The Rôle of Membranes in Cell Processes. Pop. Sci. Mo., vol. 82.

Loeb, J., 1913. Artificial Parthenogenesis and Fertilization. Chicago.

1914 Activation of Unfertilized Egg by Ultra Violet Light. Science N. S., 40, p. 680.

McClendon, J. F., 1912. The Osmotic and Surface Tension Phenomena of Living Elements and their Physiological Significance. Biol. Bull., vol. 22.

Packard, Chas. 1914 The Effect of Radium Radiation on the Fertilization of Nereis. Jour. Morph., vol. 22.

Richards, A. 1914 a Effect of X-Rays on the Rate of Cell Division in the Early Cleavages of Planorbis. Biol. Bull., xxvii.

1914 b Effect of X-Rays on Action of Certain Enzymes. Amer. Jour. Physiol., vol. xxxv.

Richards, A., and Woodward, A. E. 1915 Note on Effect of X-Radiation on Fertilizin. Biol. Bull., vol. xxviii.

THE VASOTONIC AND THE VASOREFLEX CENTRE

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I

In 1910 the writer questioned the belief that both the arterial tonus and the vasomotor reflexes are controlled by the same master cells, the so-called vasomotor centre. He pointed out that this conception was purely an hypothesis—a far-reaching hypothesis, for if the vasotonic and the vasoreflex centre are identical, the measurement of the vasomotor reflexes will reveal the condition of the apparatus for the maintenance of both functions, the tonus as well as the reflexes.

In that investigation[1] two methods were used to test the truth of the hypothesis. These methods indicated that the tonus and the reflexes were not controlled by the same nerve centre. Indeed they seemed to indicate that the bulbar cells did not modify the vasomotor reflexes. Yet the investigator expressly declined to go further than the conclusion that his work marked the speculative character of the hypothesis under examination and showed the need of further research to justify its acceptance.

This caution was well grounded. The problem in hand must at present be classed with those upon which a working decision should be made but which apparently cannot be decided by a crucial experiment. In such cases we must weigh the evidence pro and con and take the more probable side. The degree of probability depends naturally on the character of the observations and on the number of experimental methods that can be made to converge on the point attacked. In the previous

[1] W. T. Porter. This journal, 1910, xxvii, p. 276.

investigation but two methods were employed and their combined evidence was far from being conclusive. It was for these reasons that the writer reserved judgment.

The present communication brings forward a third method, the results from which agree with those from the first and second methods in that they apparently separate a vasotonic and a vasoreflex centre but differ in that the third method shows the reflexes actively affected by the condition of the vasoreflex centre.

II

The method now offered consists in applying a single reagent to the alleged single vasomotor centre. If the centre be indeed single, the changes produced by the reagent in the two functions of the centre should be in the same direction. When the reagent increases the tonus it should also increase the reflex. If both reflex and tonus are the results of the energy of one and the same nerve cell, both functions should be augmented or depressed as the energy of the cell is augmented or depressed. But curare, the reagent employed in these experiments, does not produce this reaction. It can be so administered that one of these functions is altered while the other is unaltered or is altered in the opposite direction. Curare thus separates the vasoreflex from the vasotonic function.

III

The experiments were performed on cats and rabbits. Following are typical protocols, selected from sixteen successful animals.

Experiment November 6, 1914. I. In an etherized cat the carotid blood pressure was recorded with a membrane manometer, the graduation scale of which is shown in figure 1. At 2.10 p.m. curare was given by the external jugular vein and the central end of the divided sciatic nerve was stimulated at intervals of five minutes. The secondary current was distinct when the electrodes were placed on the tongue. The sciatic reflex rose from 30 to 60 mm. while the tonus scarcely varied.

II. At 3.30 p.m. the cat had excreted the greater part of the curare and the experiment was repeated. Curare was again injected and the sciatic nerve was stimulated at ten-minute intervals. The result is

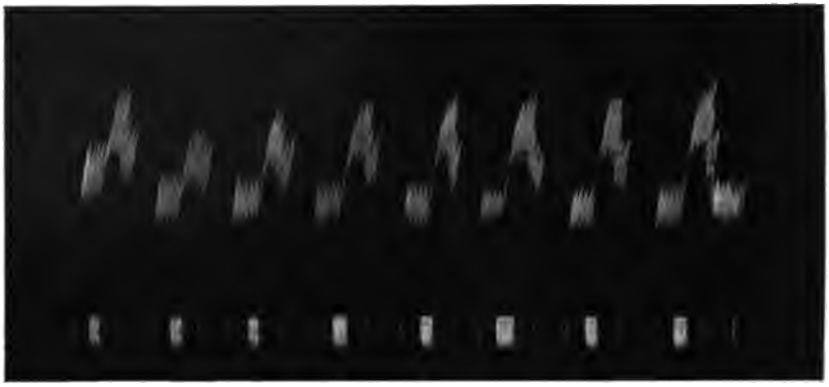

Fig. 1. About nine-tenths the original size. Carotid pressure in the cat, November 6, 1914, after the injection of curare. The sciatic nerve was stimulated at intervals of ten minutes. The reflex rise increases greatly, while the tonus remains almost unchanged.

Fig. 2. About four-fifths the original size. The carotid pressure in the rabbit after the injection of curare. Reading from left to right, the depressor nerve was stimulated at 3, 3.05, 3.15 and 3.25 p.m., November 20, 1914, and in another rabbit at 10.31, 10.51 and 11.11 a.m., November 19. The depressor reflex greatly increases, while the tonus is little changed.

shown in figure 1. The curare caused the tonus to fall from 108 to 90 mm., at which level it remained almost constant; the reflex diminished from 42 to 30 mm. and then increased to 67 mm.[2] The individual measurements in this and the two following experiments are shown in table 1.

TABLE I

The carotid blood pressure before and during stimulation of the sciatic and the depressor nerve at successive intervals of 5 or 10 minutes following the injection of curare

CAT. SCIATIC REFLEX				RABBIT. DEPRESSOR REFLEX			
Date	Before stimulation	During stimulation	Absolute rise	Date	Before stimulation	During stimulation	Absolute fall
November 6.........	110	140	30	November 19.......	90	58	32
I	110	142	32		93	39	54
	113	150	37		100	40	60
	110	151	41		108	38	60
	110	155	45		100	32	68
	111	154	43		90	32	58
	110	158	48		99	36	63
	110	150	40				
	106	148	42	November 20.......	108	90	18
	108	160	52		88	57	31
	100	160	60		90	48	42
	106	162	56		90	38	52
November 6.........	108	150	42				
II	90	120	30				
	92	138	46				
	90	142	52				
	85	141	56				
	90	143	53				
	95	141	46				
	90	157	67				

Experiment November 19, 1914. The carotid pressure of a rabbit was recorded. At 10.25 a.m. the depressor nerve was stimulated. At 10.26 curare was injected. At 10.31 and every ten minutes thereafter until 11.31 a.m., the depressor nerve was again stimulated. On the

[2] In order to shorten figure 1, the kymograph was stopped an instant after the reflex rise began and was released an instant before the maximum rise was obtained.

right hand side of figure 2 are shown the reflexes obtained at 10.31, 10.51, and 11.11 a.m.

In this experiment, the tonus remained almost constant, while the depressor reflex increased from 32 to 68 mm.

Experiment November 20, 1914. A rabbit was curarized at 2.55 p.m. and the fall in blood pressure on stimulation of the depressor nerve was measured at 3.00, 3.05, 3.15, and 3.25 p.m. The carotid pressures before stimulation were 108, 88, 90, and 90 mm. In other words, the tonus fell slightly and then remained practically constant. The depressor reflex, as shown at the left of figure 2, increased from 18 to 52 mm.

IV

These experiments show that curare may more than double the sciatic and the depressor reflex change in blood pressure while the arterial tonus is left substantially unchanged. It seems impossible to reconcile these results with the present conception of the vasomotor centre. Unless this can be done, it will be necessary to accept a vasotonic and a vasoreflex centre, related but separable.

THE EFFECT OF PARTIAL ADRENAL DEFICIENCY UPON SYMPATHETIC IRRITABILITY

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In 1904 Elliott (1) reported the observation that in a cat moribund after complete adrenal extirpation the pressor reaction to nicotin was abolished and concluded that under such circumstances the irritability of the sympathetic system is lost. He offered the suggestion that adrenal deficiency results in a corresponding deficiency in circulating epinephrin which in turn renders the sympathetic myoneual junctions incapable of transmitting impulses. In 1909 Gautrelet and Thomas (2) reported results obtained in a dog and a rabbit that supported Elliott's conclusion. Last year Hoskins and Wheelon (3) further investigated the matter. They thought that animals near the point of death are scarcely capable of giving significant information. Accordingly they studied the condition of the vasomotor mechanism in dogs during the earlier hours after ligation of both adrenals. They hoped thereby to detect the primary effect of adrenal deficiency before secondary results had obscured the picture. In such experiments they were unable to find any evidence of sympathetic depression at a time when the animals were showing marked muscular and cardial weakness. Their method of attacking the problem was based upon a supposition that the organism is not significantly affected by the loss of any quantity of adrenal tissue short of that which causes death. That idea receives a certain amount of support from such experiments as those recently reported by Crowe and Wislocki (4). These investigators found that glycosuria is caused by the manipulation of any fragment of adrenal tissue that is sufficient to maintain life,

but that equal irritation in the adrenal region in the absence of the gland tissue is without effect.

Whipple and Christman (5), however, have recently shown that partial adrenal deficiency causes a decrease in the amount of phenoltetrachlorphthalein excreted into the intestine, a result which they attribute to hepatic depression.

In the light of these results it seemed desirable to study the effect of partial adrenal deficiency upon vasomotor irritability. In order to obtain results as well marked as possible an attempt was made in the earlier experiments to reduce the adrenal tissue to the lowest amount compatible with survival. In a series of seventeen dogs one adrenal—usually the right—was destroyed completely, and at the same operation one-half to three-fifths of the other gland was similarly treated. An epidemic of distemper, added to the severity of the operation, gave a high mortality in the series but five of the animals survived. This series was supplemented by six successful cases in which the left adrenal only was destroyed.

The general methods employed in the research were the same as those previously described by Hoskins and Wheelon (6). Blood pressure from a femoral or carotid artery was recorded by means of a mercury manometer, using a reservoir cannula filled with 10 per cent sodium citrate. The reaction to a standard dose of adrenalin gave an index of the condition of the peripheral vascular structures. Similarly the reaction to nicotin indicated the degree of irritability of the sympathetic system proper. After the reactions to adrenalin and nicotin were obtained the vessels were ligated and the incision closed and dressed with a piece of gauze saturated with flexible collodion. In view of the fact that the whole procedure of setting the cannulas and closing the wound requires less than 10 minutes it did not seem worth while to attempt to surmount the difficulties of a "bloodless" technique. Aseptic precautions were taken throughout. Various methods of destroying the adrenal tissue were tried: excision, actual cautery, interstitial injections of chloroform or chromic acid and simple ligation. The injection methods were unsatisfactory in that they were hard to control. The liability to hemorrhage

and danger of injury to splanchnic nerve trunks rendered the cautery unsatisfactory. Excision of the adrenals without undue injury to nearby structures is notably difficult and time consuming. Ligation, on the other hand, is easily accomplished and is equally effective. This statement is based upon two observed facts: the reaction of the animal to ligation is characteristic of adrenal destruction and subsequent examination of the ligated glands shows that they have undergone destructive degeneration. In both these respects the present research has confirmed the observations of earlier investigators (7). The technique employed in isolating the glands was the following: The peritoneum over the adrenal was torn loose and by blunt dissection the organ was partially loosened from contiguous structures. Particularly it was sufficiently separated from the sympathetic trunks that subsequent tightening of the ligatures would not injure these. Two strands of strong linen thread were then passed together longitudinally under the gland. These were brought up, one on either side, and tied. The gland was thus completely isolated. This procedure is especially advantageous in case of the right adrenal which in the dog usually lies well under the vena cava. A bit of the dorso-lateral wall of the cava was grasped in a hemostat. Lateral traction then rolled the vein off the gland and left it fairly accessible, while offering no serious impediment to the circulation.

Parenthetically, it may be permissible to allude again to the advantages of the reservoir cannula method in routine blood pressure work. The cannula we have finally come to use with dogs is one of the ordinary arterial type in which is blown a bulb holding about 15 cc. This is filled with 10 per cent sodium citrate solution and attached directly to the manometer. The method avoids the inconvenience of developing an initial positive pressure in the system and of maintaining a cumbersome "wash-out" arrangement. When the artery clip is released an outflow of blood forces the citrate from the central stem of the cannula and partially displaces that in the bulb until pressure equilibrium is reached. The animal is thereby protected from a not uncommon accident—an intravascular injection of the anti-coagulant

when the artery is opened. Before coagulation has had time to occur, the citrate makes its way back against the blood column in adequate quantity to prevent clotting, but not enough reaches the circulation to cause any perceptible effect. This is true even though a considerable fall of blood pressure occur. It is rarely necessary to remove the cannula and dispose of a clot even in experiments lasting two hours or longer. The simplicity of the method renders it particularly advantageous for students' use in conventional blood pressure experiments. The only precaution we have found necessary is to avoid the use of too large a reservoir.

At intervals of one to eight days in various cases, after adrenal operation the blood pressure and reactions to adrenalin and nicotin were again determined. The results indicate that partial adrenal deficiency does result in sympathetic depression. Experiment No. 4 which illustrates the general outcome of the series will be described:

November 24, 1914. Dog, Female adult. Weight, 7 kilos. Cannulas set in right femoral artery and vein. Reactions determined to adrenalin 2 cc. 1–200000, nicotin 0.8 cc., 1:4000, pilocarpin 0.5 cc. 1–10000.

Laparotomy. Abdomen opened in median line. Right adrenal gland exposed and ligatures placed so as to isolate posterior half of gland—circulation of anterior half apparently not harmed. Right gland exposed and completely isolated with two ligatures. Incision closed in three layers. Excellent recovery.

November 27. Dog somewhat weak as judged by resistance to anesthetizing. Incisions in leg and belly wall clean. Cannulas set in left femoral artery and vein. Reaction to adrenalin, nicotin and pilocarpin obtained as before.

December 3. Dog weak. Incisions not well healed but apparently not purulent. Cannulas in carotid artery and external jugular vein. Reactions to adrenalin, nicotin and pilocarpin taken as before. Animal killed.

Post mortem findings: Right adrenal: Anterior half of gland apparently normal: Posterior half degenerated, largely replaced by sclerotic tissue. Left adrenal: Marked central liquifaction necrosis leaving thin superficial layer of soft brownish yellow tissue.

Right and left splanchnic nerve trunks traced through operative fields. No evidence of their having been injured in the operation.

Subsequent measurement of the tracings in this experiment showed that the original blood pressure was 146 mm. Three days later at the time of the second determination it was 132. Nine days after the adrenal ligation it was still lower, 110 mm. The nicotin reactions were respectively 50, 22 and 14 mm. The pressor reaction to adrenalin was unusually constant being exactly 40 mm. in each case.

In several instances the blood pressure reactions to small doses of pilocarpin were determined, before and after adrenal ligation. No significant differences were observed. Apparently, therefore, the lessened irritability of the sympathetic system is not shared by the para-sympathetics.

A possible source of error in such experiments is the nearness of the splanchnic nerve trunks to the adrenal glands. Injury to these nerves might well cause perturbations in the vasomotor reactions. Elliott has shown, however, that decentralization of sympathetic paths results in heightened irritability to adrenalin. If, therefore, injury to the splanchnic trunks were a significant factor in our results depression of the nicotin reaction should be accompanied by augmentation of the adrenalin reaction—a condition that ordinarily did not maintain.

No attempt was made to determine exactly the minimal quantity of adrenal tissue that must be removed to cause an appreciable loss of sympathetic irritability. Owing to individual variability in this respect a large series of experiments would probably be required to settle the point. In some cases we noted, however, that removal of one gland only was without effect, while in others a depression resulted. It is probable, therefore, that the "margin of safety" is about 50 per cent.

Considering that the vasomotor depression resulting from adrenal deficiency might conceivably be due to a reduction in the amount of circulating epinephrin the effect of slowly supplying adrenalin to the blood stream seemed worthy of investigation. Accordingly in two animals that showed well marked depression

in the reaction to nicotin dilute adrenalin was infused for half an hour into a vein. The results were surprising. Even though the infused adrenalin was producing little or no effect upon blood pressure the reaction to nicotine soon became smaller and when the rate of infusion was increased to cause a minimal pressor effect the nicotin reaction was abolished. These results tend to indicate that epinephrin deficiency is not the cause of the sympathetic depression resulting from adrenal deficiency. The phenomenon is being further investigated and results will be reported in a later communication.

For the apparent discrepancy between the previous results of Hoskins and Wheelon and those herein reported no definite explanation is offered. It would seem, however, that in their experiments the overwhelming severity of a laparotomy added to the immediate effects of total deprivation of adrenal tissue caused a primary failure of the cardiac metabolism before the sympathetic system had time to be significantly affected. Also the possibility exists that the depression of sympathetic irritability observed in the experiments herein reported is not at all specific, but merely one phase of general depression of vitality, such as occurs in Addison's disease. The sum total of available evidence seems to indicate that the essential feature of adrenal deficiency is an interference with fundamental metabolism—possibly oxidation—in which the more active tissues of the body suffer first.

SUMMARY AND CONCLUSION

From one-half to seven-tenths of the adrenal tissue was removed from dogs in various cases, at a single operation. At intervals of one to eight days after the operation the blood pressure and the vasomotor reaction to nicotin were decreased. The reaction to adrenalin was not similarly affected. Partial adrenal deficiency therefore results in a depression of the irritability of the sympathetic nervous system proper. This depression is probably only one phase of a generalized interference with fundamental metabolism.

BIBLIOGRAPHY

(1) Elliott: Journal of Physiology, 1904, xxxi, p. xx.

(2) Gautrelet et Thomas: Comptes rendus de la société de biologie, 1909, p. 388.

(3) Hoskins and Wheelon: This journal, 1914, xxxiv, p. 172.

(4) Crowe and Wislocki: Bulletin Johns Hopkins Hospital, 1914, xxv, p. 287.

(5) Whipple and Christman: Journal of Experimental Medicine, 1914, xx, p. 297.

(6) Hoskins and Wheelon: This journal, 1914, xxxiv, p. 81.

(7) Mossou et Le Play: Comptes rendus de la société de biologie, 1909, p. 36, p. 83; Allen: Glycosuria and Diabetes, Boston, 1913, p. 863; Hoskins and Wheelou, loc. cit. (3).

THE INVERSION OF RESPIRATORY WAVES IN SPHYGMOMANOMETER RECORDS OF ARTERIAL PRESSURE IN MAN

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In their work on the indirect determination of blood pressure Howell and Brush[1] showed experimentally among other things, that maximum oscillations of the sphygmomanometer lever obtain when the extra-arterial pressure of the instrument equals the intra-arterial pressure during diastole.

Since then as is well known various instruments have been devised for the express purpose of bed-side determination of diastolic as well as systolic pressure. One of the earliest of these instruments, and one which still proves to be most satisfactory in this laboratory, is the Erlanger sphygmomanometer.[2]

Those who are familiar with these instruments and with the graphic records obtained from them are also familiar with the respiratory waves which appear almost constantly in them.

During routine instruction the writer's argument for the interpretation of these respiratory waves has been somewhat as follows:

1. Observing a continuous graphic record of the sphygmomanometer with external pressure gradually falling from a point somewhat above systolic to a point somewhat below diastolic, one notes that the excursions of the writing point at first increase from smaller to greater height, then having reached a maximum fall again to smaller dimensions.

2. The region of greatest excursions in general indicates an equilibrium between the external pressure upon the artery and the internal pressure during diastole. The more the external pressure deviates

[1] Howell and Brush: Proceedings of the Massachusetts Medical Society, 1901, xviii, pp. 655–672.

[2] Erlanger, J.: This journal, 1902, xii, p. 53.

from the internal (diastolic) pressure the smaller become the excursions; the more nearly the external pressure approaches internal (diastolic) pressure the greater become the excursions (Howell and Brush, 1901).

3. With the external pressure kept constant at any given point the excursions of the sphygmomanometer lever ought also to remain constant provided no change of the internal pressure of the artery occurs. Such a condition of internal pressure however rarely obtains. The so-called respiratory waves in the graphic records are familiar evidences of this fact.

4. With the sphygmomanometer pressure set at a point near mean blood pressure the respiratory waves, as usually observed, are most pronounced. The part of this wave made up of increasing excursions must be indicative of rising internal pressure. For since the external pressure remains constant and considerably above the lowest diastolic, and since increasing excursions indicate approach toward equilibrium of the two pressures, the lower, but variable, internal pressure must be increasing toward the higher, but fixed, external pressure.

Conversely the part of the respiratory wave made up of decreasing excursions must be indicative of a falling internal pressure.

5. If together with the sphygmomanometer record the respirations of the subject are graphically recorded, then it is possible to determine what part of the respiratory wave in the blood pressure trace corresponds to the inspiratory act, what part to the expiratory act (Erlanger, 1905).[3]

With such a record one may demonstrate whether there is inspiratory rise and expiratory fall of blood pressure in the subject, or *vice versa*; or whether the changes are of a more complicated character. Indeed Erlanger and Festerling[4] have used this method in a study of the effect of respiratory movements upon blood pressure in man.

6. As a logical consequence of the above argument the following proposition ought to hold good, and ought to be borne out by experiment:

If the sphygmomanometer pressure on the arm be set at some point *below* diastolic pressure, instead of *above* as is usually done, one ought also to obtain respiratory waves in the record. *Only the waves then would appear inverted when compared with those in the record taken with external pressure above diastolic.*

[3] Erlanger: Journal of Experimental Medicine, 1905, vii, p. 713.
[4] Erlanger and Festerling: Journal of Experimental Medicine, 1912, xv, p. 370.

One ought to be able to demonstrate this inversion of respiratory waves by simply recording the respiratory movements graphically and simultaneously with the sphygmomanometer record. Care need only be taken to have the two writing points in the same vertical line.

Having determined the diastolic pressure of the subject in the usual way, one record should be taken with the external arterial pressure set above the diastolic, another record taken with the external arterial pressure set below the diastolic pressure.

In preliminary tests with students it was very gratifying to find that this inverted respiratory wave could actually be obtained, and the test was incorporated as one of the accessory exercises of the laboratory.

As will be seen below most of the subjects, so far carefully examined, show the inverted wave in their sphygmomanometer records.

The chief significance of this inverted respiratory wave, at this point of our consideration, is two-fold. (1) It demonstrates deductively the validity of the general proposition that maximum excursion of the sphygmomanometer lever (Erlanger instrument) is indicative of diastolic pressure. (2) It furnishes a method for the determination, and interpretation, of blood pressure changes in the respiratory waves of the sphygmomanometer record.

Before speaking of the experimental results the writer wishes to state, in the interests of priority, that while the idea of the inverted respiratory wave and its demonstration had been independent on his part yet he had been anticipated by Erlanger and Festerling.[5]

In the early part of the observations taken in this study the writer in conversation with one of these authors (Erlanger) discovered that they too had seen the logical consequences of Erlanger's earlier work (1905).

However, Erlanger and Festerling only observed the inverted wave upon the "artèriograph" records taken on the exposed artery of a dog. In man they were wholly unable to observe it.[6]

[5] Erlanger and Festerling: Loc. cit., pp. 380–381.

[6] Ibid., p. 384. "In the case of man it seems that the inversion of the waves of oscillation associated with respiration does not take place. . . . ."

The results here reported are therefore new in the sense that they show the presence of an inverted respiratory wave in the sphygmomanometer trace of man.

EXPERIMENTS

Method. The devices employed hardly need description beyond the statement that care was taken to avoid leaks in the closed air spaces of the transmitting tambours, tubing, etc., and to have the tambours themselves covered with rubber dam of the proper resilience.

As stated above the Erlanger sphygmomanometer was used. Whenever a larger recording surface was desired, that of an ordinary kymographion drum was substituted for the small drum supplied with the sphygmomanometer.

In the latter experiments the recording tambour transmitting the respiratory movements was supplied with a lever writing in a vertical line. Ludwig's form with jointed writing tip (used with the lever arm directed at right angles to the tangent-plane of the drum) was employed in some of the experiments. Such a writing tip, it will be remembered, traces the *chord* of the arc described by movments of the lever arm.

In other experiments a writing lever specially devised by the author was employed. This lever consists of a short writing tip suspended from the end of a thread which in turn is held on the grooved rim of a light wheel of 12 cm. radius. To prevent lateral oscillations of the writing tip it is suspended within guide posts. A smaller wheel of 12 mm. radius is rigidly attached to the axis of the larger wheel; a thread suspended from the rim of the smaller wheel is fixed to the recording tambour. The wheel is set up so that its movements are through the vertical plane. The writing tip thus records the *tangent* of the arc described by the movements transmitted from the tambour. In contradistinction to the Ludwig *chord describing* lever this instrument may be known as a *tangent recording* lever, and will be so referred to in this paper.

The advantages of a lever recording vertical lines rather than arcs are too obvious to merit further mention.

In all records where synchronous points of two or more tracings are to be determined "scratch marks" are indispensable. Accordingly no records in the present study were considered that do not bear such scratch marks.

The receiving tambour for the respiratory movements was either a pneumograph devised by Howell consisting of a rubber-bag (made of a section of inner bicycle tubing) applied directly to the chest walls. A modified Marey's sphygmoscope is inserted in the path of transmission of this apparatus. At times the pneumograph of P. Bert (simple metal cylinder with rubber dam heads), or that of Marey (steel spring plate) was used. Care was taken to apply the pneumograph to the chest-walls and upper abdomen in such manner as to enable the instrument to record promptly and faithfully the respiratory movements.

It should here be stated that in order to make the inverted respiratory wave stand out prominently upon the record the rate of respiration must be slow enough to include 6–10 heart beats, or even more. Slow, deep breathing therefore is the rule. It may be also added that the inspiratory phase should be of a duration equal to that of the expiratory phase, and if possible pauses between the two phases should be avoided.

Results. The observations upon which this study is based were made partly in the spring of 1913, partly in the fall of 1914. Many observations were made by students of the present third and second years, medical department, under the writer's supervision.

The men were of normal health and of ages varying for the most part between twenty-two and twenty-eight years.

The writer wishes to take this opportunity to thank these gentlemen who thus have assisted in the collection of material for this work. Especially does the writer wish to thank Messrs. Rice, Shipton and Stifel of the second year class for the technically perfect and beautiful records which they have kindly donated to the study.

Up to the present writing twenty-eight persons in all have been satisfactorily examined for the inverted respiratory wave. Of this number twenty persons showed an inversion of the

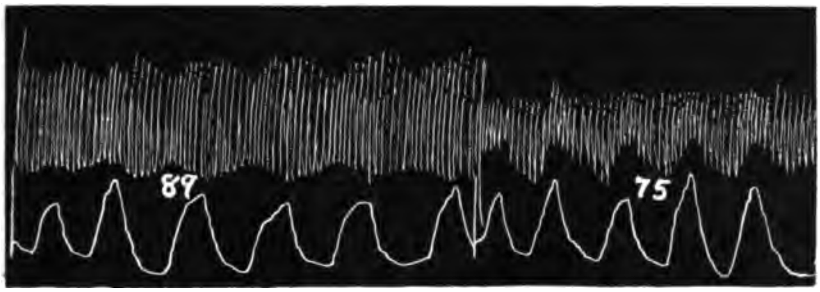


Fig. 1. Subject R. B. S. Diastolic pressure, 82 mm.; systolic pressure, 120 mm.; tangent recording respiratory lever, upstroke indicates inspiration; the numbers in the figure, 89 and 75, indicate the external pressure applied to the brachial artery during the experiment.

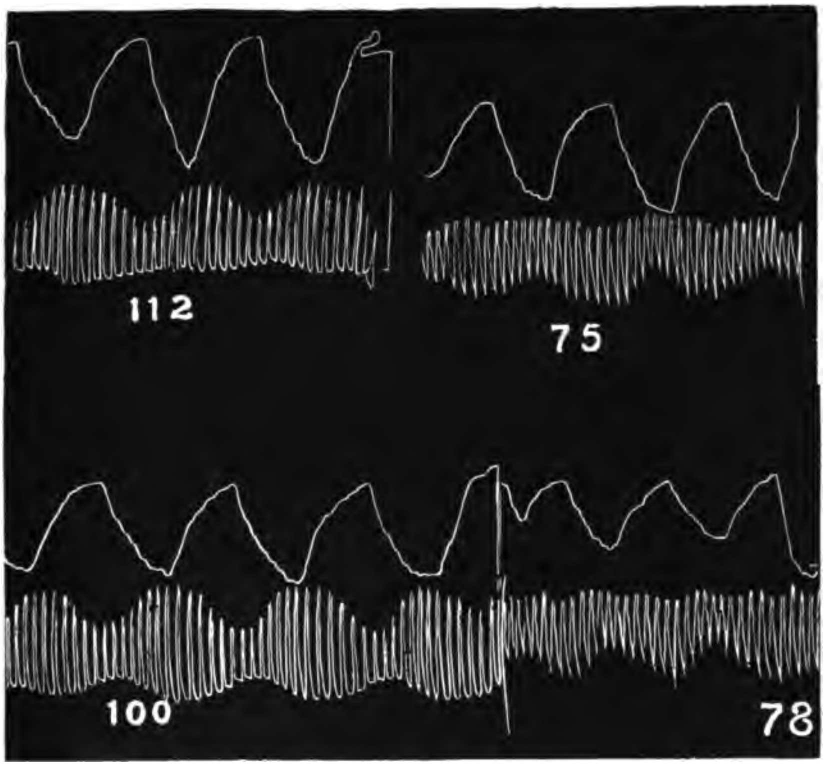


Fig. 2. Subject C. D. S. Downstroke of the respiration lever (Ludwig's lever) indicates inspiration. The numbers below each section of the tracings indicate the extra-arterial pressures applied at each reading, the pressures 100 and 112 being above, the pressures 78 and 75 being below diastolic pressure (88 mm.)

respiratory wave, and eight failed to show it. It was further observed that some individuals who showed the inverted wave at one time, at another time failed to show it. It is possible, therefore, that if the eight individuals who failed to show the wave had been examined under different conditions, they too would have shown the inverted respiratory wave.

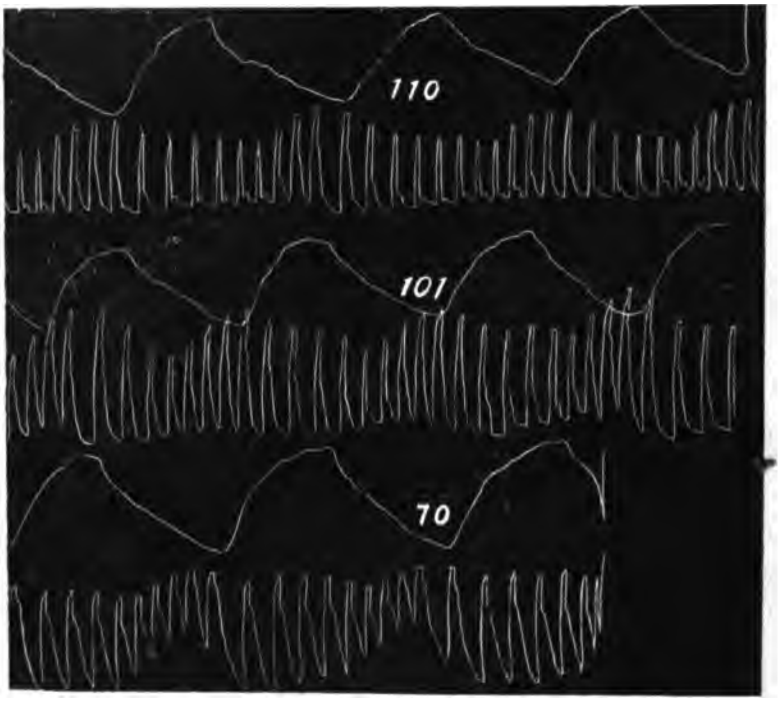


Fig. 3. Subject E. H. C. Diastolic pressure, 96 mm.; downstroke of respiration lever indicates inspiration. The numbers indicate the external pressure in the cuff of the sphygmomanometer at each of the three readings.

Indeed an analysis of the records in regard to change of heart rate and blood pressure within the respiratory cycle seems to indicate that the failure to demonstrate the inversion of the respiratory wave may be due to a certain physiological complex prevailing only at the time.

For example, of the twenty cases showing inversion of the respiratory wave eighteen could be classed as having rise of blood-pressure chiefly during inspiration, fall of blood-pressure during expiration. Of the eight cases failing to show inversion of the respiratory wave six could be classed as having fall of blood pressure chiefly during inspiration, rise of blood-pressure chiefly during expiration. From the premises stated above one ought to obtain the inverted respiratory wave regardless of the type of blood-pressure change associated with the respiration. The point evidently requires further investigation.

Specimen records showing the inversion of the respiratory wave in the sphygmomanometer are here reproduced. The inversion is seen readily if one compares sections of a record, one of which was made with the external arterial pressure above, another of which was made with the external arterial pressure below diastolic.

In figure 1, record of R. B. S., comparing the two sections shows the inversion. The one section was made with external pressures at 89, the other section was made with external pressure at 75 mm. The diastolic pressure of this individual at the time was 82 mm. Hg.

In figure 2, one compares the sections with external pressures set 112 and 75, or the sections with external pressures of 100 and 78 mm. The diastolic pressure of this individual was about 88 mm.

Again in figure 3, one compares the middle (or the upper) with the lower section. The diastolic pressure of this subject at the time was 96 mm. The external pressure on the arm in the upper section was 110, in the middle record 101 and in the lower record 70 mm.

One now will ask how much above and how much below the diastolic pressure must the pressure in the cuff be set to ensure an inversion of the respiratory wave. The answer to this would be, theoretically, not more above nor below than the amount of blood pressure change accompanying the respiration. Practically one sees in figure 1, that the two pressures between which inversion took place are pretty close together, just 7 mm. above

and 7 mm. below the diastolic pressure as determined at the time.

If one take a record with the escapement set so as to allow the pressure in the cuff to fall gradually one ought to find in the record the exact point where the inversion takes place. This has been done and is shown in figure 4. The upper sections of the record show records with external pressure constant at three different levels. Inspection will show the respiratory wave to have undergone inversion. The lower and more important part of the figure is a record of the same individual taken with external pressure gradually falling.

As will be noted the point where the inversion occurs is between 90 and 95 mm. pressure in the sphygmomanometer cuff. The inversion here, as in most cases studied, takes place at the region of last maximal oscillations.

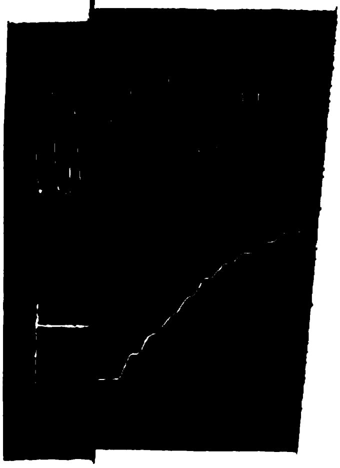
In this study, at any rate, the last maximal oscillations has always been the criterion of diastolic pressure, regardless of sounds.

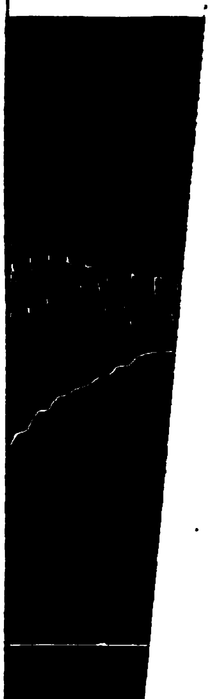
A further point is suggested by the record shown in figure 4. If the inversion of the respiratory wave in the blood-pressure trace takes place at diastolic pressure may it not be that this point of inversion itself at times could be profitably used as an additional test as to the correct diastolic pressure in man? In figure 4, at 95 mm. no inversion has yet taken place while at a point just below 90, say 88 mm., the inversion is already accomplished. Clearly in this case 90 mm. is very near the exact point of inversion. The region between 90 and 95 is the region of last maximal oscillation and therefore it is clear that the region of inversions and the region of true diastolic pressure may be one and the same in terms of pressure.

SUMMARY

1. An argument is submitted demonstrating deductively the following proposition:

In case the actual respiratory movements in man are synchronously and graphically recorded together with the sphygmomanometer trace, and with the extra-arterial pressure set at





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various points above and below the diastolic pressure, then upon inspection one ought to find an inversion of the respiratory wave in the blood-pressure trace with reference to the waves of the respiration trace itself.

This inversion of the respiratory wave in the sphygmomanometer trace ought to take place in the vicinity of diastolic pressure.

2. Graphic records were taken on twenty-eight individuals, twenty of whom clearly showed inversion of the respiratory wave in the blood-pressure trace. Reproductions of records illustrating the inverted wave are submitted in the report.

3. The inversion of the respiratory wave is not always obtained; eight of the twenty-eight individuals failed to show it. The same individual however who did not show the inversion at one time occasionally was observed to show it at another time. No satisfactory explanation of this failure can at this time be given.

4. It is suggested, and evidence is adduced showing, that the inversion of the respiratory wave in the blood pressure wave may itself at times serve as an additional test in the determination of the correct diastolic pressure in man.

THE TOXICITY OF OIL OF CHENOPODIUM[1]

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Although the oil of chenopodium has been used in medicine more than one hundred years it has as yet hardly attracted the attention of pharmacologists. The first and only record of any experimental studies on animals appeared not quite ten years ago when Brüning (1) published the results he obtained with this drug in different species of animals. His experiments indicate that even in small doses this substance may cause severe symptoms and death. The subcutaneous injection of 0.3 cc. per kilo into rabbits caused death in four days, although 1.5 cc. per kilo, given by mouth, produced salivation only. Two-tenths of a cubic centimeter per kilo, given subcutaneously to dogs, was fatal within twenty-four hours, and 0.2 to 0.4 cc. per kilo produced the same effect in five hours in frogs which received it by injection into the dorsal lymph sac. In the hen, according to his experiments, 0.5 cc. per kilo by mouth produced narcosis, paralysis and death in a few hours. Narcosis was produced in fishes by a concentration of 1:12,500 and death when the concentration was 1:8000. He has shown further, by experiments on frogs, that a more active substance which is toxic also when inhaled, may be obtained from the oil.

In the following experiments it was aimed, as far as possible to determine accurately the resistance of various animals to oil of chenopodium as well as ascaridole (2), which is a peroxide, and a product (3) derived from it, in which the two oxygens were rearranged, transforming it into a dioxide, or glycol anhydride.

[1] The results of some of the experiments were communicated before the American Society of Pharmacology and Experimental Therapeutics. Journal of Pharmacology and Experimental Therapeutics, Vol. II, p. 391.

Attention was also directed to the influence of diet and fasting and to cumulation. A very large number of experiments were performed, but only a few were selected for illustrating the typical action of these substances.

EXPERIMENTS ON RABBITS

The effects of oil of chenopodium and ascaridole, which were given by mouth and subcutaneously, varied a good deal in different individuals, the size of the dose being an important factor in determining the action. After large doses, symptoms appeared within a few minutes even when given by mouth. In some experiments in which a little more than the surely fatal dose was given the effects manifested themselves ten minutes after its introduction into the stomach, but this interval was still shorter when two or three times this amount per kilo of body weight was administered. Symptoms indicating depression of the nervous system usually appeared in one to two hours when given by mouth. The animal became somnolent and inactive, slight incoördination of the muscles of the extremities developing about the same time. Deep coma and paralysis developed later and terminated in death three or six hours after the drug was given. In other experiments tremors and mild spasms appeared first, followed by coma, convulsions and opisthotonus. In a very large proportion of experiments symptoms of a somewhat different order were observed. The initial effects were predominately those of stimulation. Within one to four hours after receiving the oil or ascaridole the animal became restless, looked as if frightened and ran wildly all over the room. Muscle tremors and incoördination became marked and were followed by convulsions which developed within several hours. In some experiments no effects were noticed until the next day. The symptoms persisted several hours and sometimes one, or even two days. The convulsions, which were clonic in character, were sometimes accompanied by peculiar cries, and were sometimes so violent that the cages in which the animals were confined were upset. Dyspnoea, opisthotonus, with cessation of respir-

ation, marked the final stages of intoxication. On autopsy the heart was found beating and continued in this condition several minutes. Although the symptoms of intoxication by the oil of chenopodium and of ascaridole were in most cases practically the same, it was noticed that in a number of experiments convulsions were more apt to follow the administration of large doses of ascaridole. The symptoms produced by the dioxide derived from ascaridole were those wholly due to depression of the nervous system and of the muscles. The reflexes after toxic doses were decreased although the conjunctival reflexes persisted even when the animals were in deep narcosis. Examination of the urine showed a very marked reduction in a number of experiments, but only rarely was albuminuria observed. The appearance of the organs in poisoning with chenopodium or ascaridole indicates that these substances are strong local irritants. The mucous membranes of the stomach and small intestines, when these substances were given by mouth, were inflamed and sometimes hemorrhagic, and the serous coat of the small intestine injected. The kidneys were congested, in rare cases hemorrhagic. The liver in most of the rabbits examined was either normal in appearance or congested. The heart was almost always injected but the lungs were normal in appearance.

The toxicity of the oil of chenopodium varied, though by no means greatly, with the mode of administration. When given by mouth 0.8 to 1.0 cc. per kilo was invariably fatal. Some animals died in four to five hours, although in some cases such a dose proved fatal within one hour. Sometimes the duration of life was longer, but seldom exceeded twenty hours. Smaller doses were rather uncertain in their effects. Thus 0.4 cc. per kilo, when introduced into the stomach of healthy, strong rabbits, produced symptoms of severe intoxication within a few hours in most of our experiments, while the duration of life varied from three and one half hours to three or four days. On the other hand, some individuals survived doses of 0.6 cc. per kilo without showing any symptoms.

The subcutaneous administration of the oil of chenopodium proved to be somewhat more toxic, 0.3 to 0.4 cc. per kilo being

invariably fatal. The appearance of symptoms in these experiments varied considerably. In one experiment marked effects were observed one hour after injection, but more frequently the onset of symptoms was delayed a good deal longer. At least four hours may pass in some individuals after injection without the appearance of any signs of intoxication. This was also observed in experiments with the subcutaneous injection of ascaridole. The duration of life was usually one day, but in some experiments it was only five hours; in others again it was three days. Quantities under 0.3 cc. per kilo were not followed by any symptoms.

Ascaridole, which is the active principle of the oil of chenopodium, was tested in the same way as the oil. Its activity was found to be decidedly greater. One-half of a cubic centimeter per kilo given by mouth produced, in our experiments, violent convulsions, paralysis, and death within one to two hours. Such a dose, it may be observed, was always fatal. A dose of 0.3 cc. per kilo was fatal for some rabbits but was well borne by others. The lethal dose of ascaridole by subcutaneous injection was 0.2 cc. per kilo although some individuals survived the dose. Smaller doses also caused death, but a much larger number survived.

Of considerable interest is the observation that subminimum ineffective doses when repeated one and two, and even three days later, proved to be fatal. This was found to be the case whether the drug was given by mouth or subcutaneously in experiments with the oil, as well as with the active principle, thus indicating cumulative action. The experiments on rabbits which were carried out with the dioxide derived from ascaridole showed that this body was much less active than ascaridole as 0.6 cc. per kilo given by subcutaneous injection failed to produce symptoms. Nine-tenths of a cubic centimeter produced deep narcosis from which the animal recovered, however, in the course of about twenty-four hours.

Rabbit 1593. Belgian Female.
November 21. Weight, 1610 grams.
November 23. Weight, 1665 grams.

9.50 a.m. 1.7 cc. oil chenopodium in water administered by mouth.

10.00 a.m. Somnolent. Ten minutes later symptoms of intoxication well marked. Rabbit comatose and paralyzed.

10.15 a.m. to 10.20 a.m. 16 cc. cottonseed oil given subcutaneously and 22 cc. injected into peritoneal cavity. No convulsions were observed at any time.

12.40 p.m. Died.

Rabbit 1559. Black Female. Weight, 1330 grams.

November 13, 1914. Urine albumin and sugar, negative.

11.12 a.m. 0.8 cc. (0.6 cc. per kilo) oil of chenopodium given by mouth.

12.30 p.m. Muscle tremors.

2.00 p.m. Muscle tremors marked; incoördination.

November 14, 1914. 9.00 a.m. Albumin—none. Reduction—none. Rabbit lying in cage paralyzed.

November 15. Condition worse.

November 16. Dead.

Rabbit 1558. White Female. Weight, 1735 grams.

November 16, 1914. 9.50 a.m. 1.4 cc. oil of chenopodium given by mouth.

1.00 p.m. Mild spasms, muscle tremors and paralysis.

1.30 p.m. Lay in cage as if in comatose condition.

1.50 p.m. Irregular and well marked convulsion preceded by cries. Respiration was suspended about one-quarter of a minute but gradually returned.

2.00 p.m. Convulsions and death. About one minute later thorax opened, heart was still beating but was very weak.

Rabbit 1622. Belgian Female. Good condition. Weight, 2050 grams.

December 14, 1914. 11.00 a.m. Received 0.8 cc. per kilo oil of chenopodium emulsifed in 10 cc. saline acacia with a few drops of sodium carbonate. Administered by stomach tube.

12.40 p.m. Muscle tremors very marked, thoracic muscles especially twitching vigorously, but no coma.

1.10 p.m. Muscular twitching more marked.

1.12 p.m. Ataxia of anterior extremities. Rabbit fell over on left side. Head retracted. Spasms of posterior extremities at frequent intervals.

2.30 p.m. Died.

Autopsy: Gastric mucosa slightly hyperemic. Contents smelled of chenopodium. Duodenal mucosa, punctate hemorrhages. Serous coat

injected. Liver congested. Kidney showed hemorrhages of cortex. Bladder distended with urine. Heart injected, in diastole. Lungs inflated but color normal.

Rabbit 542. Belgian Female. Weight, 1725 grams.

December 16. 12.30 p.m. 0.5 cc. oil of chenopodium given subcutaneously. No symptoms observed up to 5 p.m.

December 17. 2.00 p.m. Convulsions. Stood on hind legs and fell over on her back, followed by forced movements. Tremors and restlessness during intervals between convulsions.

4.00 p.m. Quiet.

December 18. 3.00 p.m. Complete paralysis. Passed an enormous amount of feces which was normal in appearance.

December 19. 12.00 noon. Paralyzed and comatose.

Rabbit 615. White and Gray Male. Diet, Oats. Weight, 1420 grams.

May 26, 1911. 11.40 a.m. 0.7 cc. ascaridole administered by mouth.

12.30 p.m. Symptoms appeared—tremors.

1.25 p.m. Coma—opisthotonus. Posterior extremities extended; anterior extremities paralyzed; dyspnoea.

1.30 p.m. Convulsion, accompanied by peculiar cries; marked opisthotonus, and cessation of respiration, but respiration soon returned.

1.40 p.m. Severe convulsions. Rabbit cried and threw itself about on holder. Respiration ceased but soon returned.

1.45 p.m. Violent convulsions; short duration.

1.55 p.m. Violent convulsion; survived.

2.00 p.m. Convulsions with peculiar cries; died in attack.

Rabbit 1561. Gray Male. Diet, Oats. Weight, 1660 grams.

November 12, 1914. Albumin—trace.

1.20 p.m. 0.85 cc. ascaridole given by mouth.

3.00 p.m. Violent convulsions, repeated attacks. No increase of reflexes.

5.00 p.m. Comatose and paralyzed.

November 13. 9.00 a.m. Dead. Urine passed after injection, trace albumin. Reduction heavy.

Autopsy: A few punctate hemorrhages into gastric mucosa which was very pale. Small intestine injected, mucosa red. Kidneys and liver congested.

Rabbit 1549. Belgian Male. Weight, 1000 grams.

November 13, 1914. 2.16 p.m. Injected 1 cc. dioxide derived from ascaridole subcutaneously.

3.00 p.m. Lay in cage unconscious. Reflexes of pupils good.

November 14, 1914. 9.00 p.m. Lay in cage unconscious, apparently comatose, paralyzed. Conjunctival reflexes good, respiration slow.

11.00 a.m. No change.

12.30 p.m. Dead.

Autopsy: Urine in bladder contained a good deal of albumin and showed marked reduction.

EXPERIMENTS ON GUINEA PIGS

The oil of chenopodium, as well as its active principle, ascaridole were employed. The toxic effects first manifested themselves by the appearance of muscle tremors and spasms of the head and neck which were soon followed by mental depression, the animal becoming dull and apathetic. Respiration became slower, and later marked dyspnoea developed. As the stage of intoxication advanced, coma and paralysis were observed Occasionally violent convulsions, almost tetanic in character, were noticed. These were frequently short with intermissions, but in some cases lasted several hours. The time of onset of symptoms usually varied a good deal. It was noticed in a number of experiments that as long as five to six hours elapsed between the subcutaneous administration of the drug and the development of symptoms which persisted at least thirty to forty hours. In other cases severe symptoms developed within two and one-half hours after the same dose per kilo. The toxicity also varied in different individuals. The smallest fatal dose was 0.2 cc. per kilo by subcutaneous injection, the duration of life being less than twenty-four hours; on the other hand larger amounts in proportion to body weight were given without producing any symptoms. The subcutaneous injection of 0.4 cc. per kilo was followed by symptoms within about five hours and death within twenty-four hours, or less. One case may be recorded in which a dose of 0.5 cc. per kilo was survived. The resistance to chenopodium when given by mouth was at least twice as great as when given by subcutaneous injection. Symptoms developed in these experiments approximately three to four hours after the feeding of the drug. One-half of a cubic centimeter per kilo caused death

in two experiments; in one of these when the dose was repeated after an interval of nine days. In three other experiments the introduction of a single dose, 0.5 cc. per kilo, of the oil of chenopodium into the stomach did not produce any symptoms, and the animal survived. A considerable loss of weight, amounting to 15 or 20 per cent, was observed after subminimum doses of chenopodium or ascaridole, whether given by mouth or injected subcutaneously. Ascaridole was found to be much more active than the oil itself. The subcutaneous injection of 0.25–0.27 cc. per kilo was invariably fatal within twenty-four hours. A dose of 0.1 cc. per kilo, given as a 10 per cent solution in neutral olive oil, caused paralysis and coma in twenty-four hours and death in less than forty hours in one experiment. In another experiment such a dose had no visible effect. Oil of chenopodium, from which the ascaridole was obtained, and tested on guinea pigs, failed to produce symptoms in doses of 0.1 cc. per kilo administered subcutaneously. On autopsy signs of irritation of the mucous membrane of the intestine and injection of the blood vessels were very pronounced. The heart was also injected and much darker in color than normal, being almost chocolate colored. This condition was observed in animals to which oil of chenopodium was administered by mouth as well as subcutaneously.

Guinea Pig 192. Male White and Black. Weight, 915 grams.

November 17, 1914. 10.40 a.m. 1 cc. oil of chenopodium administered in olive oil by mouth.

11.15 a.m. No symptoms.

1.40 p.m. No symptoms.

2.00 p.m. Muscle tremors, weakness of extremities.

2.15 p.m. Spasms of muscles of neck. Chin rested on floor. Posterior extremities weak.

3.15 p.m. Coma and paralysis.

4.00 p.m. Dyspnoea, coma, paralysis.

November 18. 9.00 a.m. Found dead.

Guinea Pig 196. Male Black and Yellow. Weight, 955 grams.

November 18, 1914. 11.35 a.m. 5 cc. 10 per cent oil of chenopodium administered in olive oil by mouth.

2.45 p.m. No symptoms.

4.30 p.m. Condition good: No symptoms.

November 19. 9.00 a.m. Alive: No symptoms.

November 27. Condition good.

4.10 p.m. 0.4 cc. oil of chenopodium given by mouth, followed immediately by 10 cc. olive oil.

5.00 p.m. No symptoms.

November 28. 9.00 a.m. Found dead.

Autopsy: Small intestine markedly injected. Mucous membrane inflamed and covered with mucus. Gastric mucosa slightly congested. Considerable oil found in stomach and intestine. Odor of chenopodium very distinct. Heart injected and discolored.

Guinea Pig 197. Male White and Yellow. Weight, 880 grams.

November 18, 1914. 11.00 a.m. 0.5 cc. oil of chenopodium administered by mouth.

2.45 p.m. General tremors, but able to walk well.

4.30 p.m. Weakness of posterior extremities. No other symptoms.

November 19. 9.00 a.m. Found dead.

Autopsy: Findings the same as No. 200.

Guinea Pig 202. Male Black. Diet, Hay and Grass. Weight, 760 grams.

November 19, 1914. 1.59 p.m. 0.8 cc. 10 per cent oil of chenopodium in olive oil administered subcutaneously. Under observation all afternoon. No symptoms.

November 20. No symptoms all day.

November 21. No symptoms.

November 27. Condition good. Weight, 605 grams.

4.05 p.m. 0.35 cc. oil of chenopodium administered by mouth through stomach tube.

December 3. Alive, no symptoms.

Guinea Pig 200. Female Black and Brown. Weight, 755 grams.

November 18, 1914. 12.20 p.m. 3 cc. 10 per cent oil of chenopodium in olive oil administered subcutaneously.

2.45 p.m. Convulsion—frequent attacks and well marked. Short intermission: Able to use legs, that is, able to stand and walk during intervals between attacks.

4.30 p.m. Prolonged convulsions. Attacks every few seconds.

November 19. 9.00 a.m. Found dead.

Autopsy: Heart almost chocolate colored—injected. No odor of chenopodium. Liver congested. Gall bladder distended with clear

bile which was straw colored and odorless. Stomach inflamed in spots. Small intestine much injected and inflamed. Hemorrhagic.

Guinea Pig 195. Male Mole Color and White. Weight, 800 grams.

November 18, 1914. 11.15 a.m. 2 cc. 10 per cent oil of chenopodium in olive oil administered subcutaneously.

2.45 p.m. No symptoms.

3.30 p.m. Paralyzed, but able to crawl with difficulty.

4.30 p.m. Paralyzed, spasms of extremities occurred periodically. Rabbit lay on its side.

November 19. 9.00 a.m. Found dead.

Guinea Pig 198. Male White and Yellow and Brown. Weight, 780 grams.

November 18, 1914. 11.55 a.m. 1.5 cc. oil of chenopodium in olive oil administered subcutaneously.

2.45 p.m. No symptoms.

4.30 p.m. No symptoms.

November 19. 9.00 a.m. Found dead.

EXPERIMENTS ON CATS

When the oil of chenopodium, which was given by mouth and subcutaneously, was administered in sufficient quantity, salivation was the first symptom to appear and frequently lasted until the end of the experiment. Later, depression of the higher nervous centers set in. The animal became somnolent and appeared to be in a semi-conscious condition, even after moderate doses, the head drooping, sometimes the nose touching the floor of the cage, the spine bent and muscular atony being quite marked. Vomiting was observed in a good many cases whether it was given by mouth or subcutaneously, but the time of the occurrence varied. In some subjects it was observed about an hour after the administration, but in others it was delayed for several hours. Incoördination, weakness of extremities, tremors, and convulsions, clonic in character developed in all experiments when the dose was sufficiently large, in which case symptoms of severe intoxication developed within one hour. Convulsions may last one or several days and may become violent. In some experiments convulsions were absent but coma and paralysis developed on the same day, or the day

following the administration of the chenopodium. The resistance in well fed subjects varied a good deal. About 0.6 cc. per kilo of the oil when given by mouth was fatal in every case within eighteen to twenty-four hours. In one cat the duration of life was nearly two days. The administration of 0.4 cc. was followed by symptoms, varying in intensity from somnolence and salivation to violent convulsions and coma. Only one cat survived this dose. Two-tenths to 0.25 cc. of the oil per kilo were toxic; but 0.25 cc. per kilo was fatal in one case. In several experiments, however, the administration of 0.2 cc. per kilo to well fed cats failed to produce any symptoms even when the dose was repeated three days later. When 0.16 to 0.2 cc. oil of chenopodium was fed to cats after they were allowed to fast from four to six days, well marked symptoms developed in all and were fatal in some cases, causing death in less than twenty hours. A second dose given two days after the first, that was only mildly toxic, caused severe symptoms within three hours and the animal died during the night. A smaller dose was not fatal, though one-tenth of a cubic centimeter produced severe symptoms in nearly all of our experiments on starving cats. Attention may be directed, however, to two experiments in which 0.3 cc. per kilo of oil of chenopodium, fed to cats which fasted five days, produced somnolence for about one day, from which they recovered, no other effects having been observed. The drug in this case, however, was given in 5 cc. olive oil instead of being mixed with water or an aqueous solution of gum acacia, as in all the other experiments on cats. As will be seen later, oils and fats probably reduce the toxicity of the chenopodium. That its effects may also be cumulative in cats was also shown in these experiments. The increased susceptibility may last several days as in Experiment 64. The changes observed on post mortem examination were those due entirely to circulatory disturbance. The heart was enlarged and the coronary vessels injected. The kidneys were also enlarged and congested, the liver showed congestion and hyperemia, but this was never very marked. No noteworthy changes could be found either in the stomach or in the intestines.

Cat 63. Tiger Female. Well-fed. Weight, 1950 grams.

December 6. 0.5 cc. oil chenopodium administered by mouth in 5 cc. water.

December 7. Cat vomited during the night; paralyzed; general tremors, coma, reflexes increased. Died in the afternoon of December 7.

Cat 64. Black and White Female. Weight, 2370 grams.

December 6. 0.5 cc. oil of chenopodium administered in 5 cc. water by mouth.

December 7. Cat vomited during the night.

December 12. In good condition. No symptoms. Weight, 2410 grams.

4.00 p.m. 0.5 cc. oil of chenopodium given by mouth in 5 cc. water.

5.00 p.m. Salivation. No other symptoms.

December 13. 9.00 a.m. Lay in cage, paretic.

1.00 p.m. Struggled, attempted to rise. No appetite—refused to eat meat placed before it. Died.

(Note): Cat was fed December 11. No food eaten December 12.

Cat 281. Black Female. Well-fed. Weight, 3200 grams.

November 18. 10.40 a.m. 1.3 cc. oil of chenopodium in water given by mouth.

Symptoms: Mental depression and weakness of posterior extremities appeared a few hours after the oil was given.

November 19. Under observation all day. Frequent attacks of clonic convulsions which became almost violent at times, cat crying frequently during the attacks.

November 20. 9.00 a.m. Convulsions still observed but not so violent. Cat looked less depressed.

November 23. Died about 12 noon.

Autopsy: Cat 281. Liver congested, kidneys enlarged and slightly congested in medulla. Heart congested. Blood vessels all over body injected. No other noteworthy changes.

Cat 282. Female Tiger. Well-fed. Weight, 3075 grams.

November 18. 10.55 a.m. 1.2 cc. oil of chenopodium in water given by stomach tube.

1.25 p.m. Salivation present but no other symptoms.

4.30 p.m. Somnolent. When placed on the floor jumped up suddenly, behaved as if excited.

November 19. Under observation all day. Salivation occasionally. Muscle tremors in posterior extremities, but not paralysis. Condition good.

Cat 279. Black Female. Well-fed. Weight, 2570 grams.

November 17. 11.55 a.m. 0.5 cc. oil of chenopodium in water administered by mouth through stomach tube. Weakness of posterior extremities noticed after several hours.

November 18, 19, 20. No symptoms, appetite good.

November 20. 11.40 a.m. 0.5 cc. oil of chenopodium administered in water by mouth through stomach tube.

3.15 p.m. No symptoms. Survived.

Cat 274. Female Tiger.

November 14. Food withdrawn.

November 16. Weight 1546 grams.

November 18. Weight, 1450 grams.

10.00 a.m. 0.3 oil of chenopodium in water given by mouth through stomach tube.

12.00 p.m. Head bent, drooping, looked sleepy.

November 19. Under observation all day. Somnolent but no other symptoms.

November 20. 9.00 a.m. Condition good, no symptoms.

12.15 a.m. 0.25 cc. oil of chenopodium given by mouth in olive oil.

3.15 p.m. Symptoms marked. Cat cried. Incoördination marked. Refused to get up. Looked severely poisoned.

November 21, 9.00 a.m. Found dead.

Cat 313. Gray and White Female. Weight, 2380 grams.

December 26. 11.30 a.m. Fed 100 grams meat.

1.50 p.m. 0.5 cc. oil of chenopodium injected subcutaneously in back.

3.30 p.m. No symptoms. Animal active and about normal.

3.40 p.m. Vomited and depressed. Lived less than forty-three hours.

EXPERIMENTS ON DOGS

Vomiting, which occurred one to four hours after the administration of the oil of chenopodium, was the first symptom observed when a sufficient quantity was given by mouth or subcutaneously. After large doses, 1.25 to 2.5 cc. per kilo, injected subcutaneously, coma and paralysis also developed, usually within two hours, terminating in death in about six hours. The symptoms were somewhat different after smaller doses. Vomiting and salivation occurred as in the experiments with large

doses, but these were followed by general depression and muscular incoördination. Later, coma and convulsions were also observed and persisted for two or three days. The minimum lethal dose, when given by subcutaneous injection, is about 0.3 to 0.4 cc. per kilo. Smaller doses, 0.2 cc. per kilo, never produced any symptoms; such amounts, however, when repeated after an interval of twenty-four hours, were fatal. That much larger doses may be given by mouth is shown in Experiment 203. It will also be noticed that there was no evidence of cumulative action in this case. Post mortem examination showed the heart to be enlarged and distended, and the coronary vessels injected. There was also enlargement of the kidneys which were congested. The liver was injected and congested, but no other noteworthy changes of the abdominal organs were noticed.

Puppy 204. Weight, 1570 grams.

November 25. 9.39 a.m. 2 cc. oil of chenopodium given subcutaneously.

10.45 a.m. Food vomited; salivation; animal depressed. Able to walk but gait stiff.

4.20 p.m. Dying.

November 26. 10.00 a.m. Found dead.

Puppy 207. Brown Female. Weight, 1550 grams.

November 25. 12.05 p.m. 0.3 cc. oil of chenopodium administered subcutaneously.

4.20 p.m. No symptoms, condition good.

November 26. 10.00 a.m. No symptoms. 0.3 cc. oil of chenopodium administered subcutaneously.

11.00 a.m. Salivation, and animal quiet. Was very noisy before injection.

November 27. 9.00 a.m. Symptoms of severe intoxication; coma and spasms.

November 28. 9.00 a.m. Found dead.

Dog 203. White Female. Weight, 4.4 kilos.

November 21. 1.50 p.m. Received 2 cc. oil of chenopodium by mouth in water by stomach tube.

4.25 p.m. No symptoms.

November 23. 9.00 a.m. Lively, general condition good.

11.50 a.m. Received 2 cc. oil of chenopodium in water and a few drops of olive oil.

12.30 p.m. Food given was vomited. No symptoms developed. Under observation several days.

Dog 212. Black Female. Weight, 10.4 kilos.

December 10, 1914. 10.55 a.m. 3.2 cc. oil of chenopodium injected subcutaneously.

11.15 a.m. No symptoms.

11.40 a.m. No symptoms. Ate meat.

1.00 p.m. No symptoms.

2.00 p.m. No symptoms.

3.00 p.m. Slight incoördination present.

3.05 p.m. Vomited.

4.50 p.m. Somnolent, gait unsteady, but able to walk.

December 11. 9.00 a.m. Looked depressed, laid down and refused to get up, unable apparently to raise herself. Was picked up and made to stand on legs a few seconds; unable to walk, incoördination being very marked in hind legs.

10.30 a.m. Lay in comatose condition; convulsion present.

12.10 a.m. Struggled. Had spasms and cried as if in pain.

December 12. 9.00 a.m. Dead.

Dog 215. Brown Female. Weight, 8.6 kilos.

December 26. 11.30 a.m. Received about 200 grams meat.

1.45 p.m. Received 5.2 cc. oil of chenopodium emulsified with 20 cc. 5 per cent gum acacia and a few drops of sodium carbonate by mouth.

2.15 p.m. Vomited. Salivation. Marked incoördination present. Also narcosis from which dog was aroused with difficulty. Breathing deep, 25 per minute.

4.00 p.m. Deep narcosis. Profuse salivation. Dog seemed to suffer pain. Lived less than forty-three hours.

DISCUSSION

Although the symptoms produced by oil of chenopodium or ascaridole were in the main very similar in all the animals upon which it has been tested, there were nevertheless some differences which may be pointed out with advantage. The guinea pig responded more frequently by showing muscle tremors at first, then convulsions and coma, while in cats and dogs symptoms of depression of the nerve centers were the first effects observed.

In the rabbit the symptoms varied a good deal in different individuals. Narcosis in some and excitement in others were the predominant symptoms observed first. In some animals narcosis was the only symptom when a sufficient amount was administered. In dogs and cats the depressing effect on the nervous system was observed shortly after the subcutaneous administration, followed by deep coma and spasms, which were rather mild by comparison with those in guinea pigs and rabbits. The course of intoxication when the dose was not very large was usually prolonged in all of the animals, lasting in some as long as three and four days.

A comparison of the resistance of different animals shows that the differences are by no means striking, the substance being about equally toxic for the rabbit, guinea pig, and dog, but somewhat smaller doses were required to produce the same effect in cats. As already stated, 0.25 cc. of oil of chenopodium per kilo by mouth produced death in one cat. Approximately twice this dose caused vomiting only in dogs. Such low resistance in the cat may, however, be regarded as exceptional. In most cases 0.4 cc. per kilo may be safely regarded as the lethal dose for the cat, when given by mouth and 0.2 cc. as the fatal dose by subcutaneous injection. Rabbits likewise differed in their behavior toward chenopodium, 0.4 cc. per kilo by mouth being fatal in most cases, but this dose and even 0.6 cc. per kilo were well borne by some individuals. This would indicate that their resistance in general is greater than that of cats. Besides, the dose by subcutaneous injection was distinctly smaller in cats. The toxicity was the same for guinea pigs when they were fed by mouth, but it was distinctly greater when given subcutaneously. It may be remarked in this connection that the difference in resistance when given by mouth and subcutaneously varied considerably in different animals. It will be recalled that in guinea pigs the minimum lethal dose by mouth was fully twice as much as by subcutaneous injection. The case was the same in dogs and in cats, but in rabbits the surely fatal dose by subcutaneous injection was the same as the minimum fatal dose by mouth. This may be explained by as-

suming that the rate of absorption is probably not much different. Indeed, the rapid appearance of symptoms after larger doses when given by mouth indicate that such is probably the case in rabbits, but absorption alone does not explain it, since in the guinea pig symptoms appeared about the same time whether given by mouth or subcutaneously. Some other factors probably enter in the determination of the greater toxicity by subcutaneous injection. May it not be due to detoxication by the intestinal contents? In a number of experiments the overdistention of the gall bladder was noticed at autopsy in the guinea pigs, but not in the rabbits. Perhaps increased secretion of the bile diminishes the toxicity of chenopodium. Detoxication of some of the essential oils by glycuronic acid was claimed by Hildebrandt (7), Matzel (8) and others. Perhaps the oil of chenopodium forms a less toxic compound with the glycuronic acid of the bile.

The effect of the nutritional condition of the animal on the toxicity of chenopodium was very strikingly shown by our experiments on starving cats as four or five days fasting exerted a marked increase in the toxicity of chenopodium. Two-tenths of a cubic centimeter per kilo, administered by mouth, produced death, and 0.1 cc. caused, in nearly all cases, severe symptoms, though recovery was the rule. Poorly nourished animals, such as were frequently brought into the laboratory, which were given chenopodium shortly after their arrival, showed a markedly lower resistance than animals which had been some time in the laboratory and were well fed. The increase of fat in the blood in starvation and the loss of fat from the tissues which occurs in this condition were probably responsible for the decrease of the resistance to chenopodium since a larger amount of it would thus be transported to the central nervous system and other organs instead of being distributed among the lipoids of the body. Support for this view is furnished by experiments we carried out on cats and rabbits in which the effects of various fixed oils on the toxicity of chenopodium were tested.

Examination of the results of the experiments on cats presented in Table 1 shows that in four experiments with 0.4 cc. oil of

TABLE I

Effect of olive oil on toxicity of chenopodium—Experiments on cats

	EXP. NO.	WEIGHT	CHEMOPODIUM PER KILO	SYMPTOMS	DURATION OF LIFE	REMARKS
		grams	*cc.*			
Cats fasted several days before experiment	275	2000	0.3	Slight 2 hours	Survived	15 cc. olive oil given with chenopodium
	278	2100	0.3	Severe	23 hours	Chen. given with water
	290	1710	0.4	1 hour vomiting	4 days	25 cc. olive oil. Vomiting oniy effect noted.
	291	3035	0.4	Slight	Survived	
	297	4040	0.4	None	Survived	Olive oil 30 cc. with chenopodium
	298	2425	0.4	1 hour severe	About 20 hours	Chen. given in 25 cc. 10% sol. gum acacia
	295	1250	0.4		About 6 hours	Chen. given in 25 cc. gum acacia. Cat emaciated—general condition poor. Coma and conv.
	296	1700	0.4		do	do in olive oil
	292	2465	0.6	Slight	Survived	Chen. in water followed by 30 cc. olive oil.
	289	2010	0.5	1 hour severe	Survived 2 days	Chen. in water followed by 30 cc. olive oil. Coma and paralysis 4½ hrs. Violent conv. next day
	293	3720	0.6	None	2 days	Chen. in 40 cc. olive oil
	294	2925	0.5	1 hour severe.		Chen. in 30 cc. 10% sol. gum acacia. Conv. in 1 hr.

chenopodium, given with olive oil, two survived without showing any symptoms of poisoning. One was found dead four days later but never exhibited any signs of intoxication, while another died after six or seven hours, with typical symptoms of poisoning by oil of chenopodium. This cat, it may be remarked, received chenopodium shortly after it was brought to the laboratory and was emaciated and seemed to be in poor condition generally.

It may be safely concluded, therefore, that such a dose does not produce death when given with fixed oils. It certainly does not cause acute effects, while it will be recalled that 0.4 cc. per kilo fed in water or with acacia was always toxic and was seldom survived. Furthermore, when olive oil was given even a larger dose, 0.6 cc. per kilo, which was invariably fatal when given with acacia or in water, produced in one case slight symptoms only, and the animal survived (292).

Experiments 275 and 278 likewise illustrate the neutralizing effect of olive oil. Both cats were treated in precisely the same way. After having been allowed to fast the same length of time they received 0.3 cc. of the oil of chenopodium by mouth but no effect was noticed as a result of the treatment. A second dose of the same size was given later in 15 cc. olive oil to one and to the other a dose mixed with water which served as a control. As shown in the table, the latter developed symptoms of severe intoxication in two hours and lived twenty-three hours while its mate survived without any appreciable effect.

In experiments on rabbits similar results were obtained. Although the fixed oils failed in some cases to modify the action of chenopodium, the results obtained indicate that the toxicity of chenopodium is undoubtedly decreased by glycerides. As stated above, 0.4 cc. of chenopodium, given by mouth, is often fatal, but some animals, however, may survive as much as 0.6 cc. per kilo. None survived a dose of 0.8 cc. per kilo. Such a dose is, therefore, surely fatal. We found, on the other hand, that 0.4 cc. chenopodium per kilo was never acutely fatal to rabbits which were given cocoanut or cottonseed oil several hours or one day previously. Six-tenths of a cubic centimeter per kilo of oil of chenopodium was fatal to some rabbits but others

survived such a dose. Evidence of detoxicating action was also obtained in experiments with larger doses. It was found that 0.8 to 1.0 cc. per kilo, given in an aqueous solution of acacia as an emulsion caused death in one to five hours. Some animals lived one day. The duration of life in three experiments when the injection of cottonseed oil subcutaneously or into the peritoneal cavity was followed by 0.8 to 1.0 cc. chenopodium, was fourteen to twenty-four hours. In three others similarly treated one cubic centimeter per kilo failed to produce any symptoms in one rabbit, another lived five days, a third, which received 0.8 cc. per kilo, lived nine days. It is evident, therefore, that acute symptoms may be suppressed by simultaneous, or even previous, administration of the fixed oils. It is doubtful whether the fatal outcome may be ascribed to the chenopodium per se. It is more likely that the death was due to loss of appetite produced by it, for in the cats, which did not suffer any loss of appetite, no after effects were observed, while in 290, which refused food, the outcome was fatal. The detoxicating effect of fat is probably due to the solubility of the chenopodium in fixed oils which led Nerking (9) to assume on the basis of the Meyer-Overton theory that anesthetics may be detoxicated by lipoids. He found that if a sufficient amount of lecithin is introduced into the circulation of animals in deep narcosis, the effects of the anesthetic may disappear promptly, also that a larger amount of it is necessary to produce anesthesia if the animal received a previous injection of lecithin. The results of Nerking were disputed by Kramer (10), who repeated the experiment. Our findings, however, would seem rather to support Nerking's conclusions.

Evidence of detoxication by the presence of large amounts of glycogen in the body was also obtained in some of our experiments, as shown in the following abbreviated protocol.

Rabbit 641, diet oats, weight 1375 grams. 0.5 cc. ascaridole per kilo. Found dead three hours later.

Rabbit 615, diet oats, weight 1420 grams. 0.5 cc. ascaridole per kilo. Symptoms appeared within one hour. Died two hours twenty minutes after receiving ascaridole.

Rabbit 640, diet carrots, weight, 1540 grams. 0.5 cc. ascaridole per kilo. Spasms in two hours. Survived.

That the essential oils may be detoxified by glycuronic acid has been shown by Schmiedeberg and Meyer (11), Hildebrandt (12) and later by Matzel (13). Mayer (14) and Hildebrandt (15) found that much larger amounts of glycuronic acid were obtained after feeding grape sugar or cane sugar which might thus explain the effect of chenopodium or ascaridole in starvation. The different behavior of chenopodium in starvation and when administered after feeding a rich carbohydrate diet may be explained therefore by assuming that under these conditions the variation in the amount of glycogen is accompanied by a difference in the quantity of glycuronic acid formed. The formation of glycuronic acid from the glycerin in fats as suggested by Neurberg (16) may also explain in part at least the neutralization of the effects of chenopodium by the glycerides.

Attention has already been called to the cumulative effect of chenopodium. A more complete survey of the data as presented in Table II indicates very clearly that when given even at intervals of several days, symptoms and sometimes death was produced by doses considerably below the minimum toxic amounts. Thus it will be noticed that 0.25 to 0.3 cc. ascaridole per kilo given by mouth caused death after the fifth and even after the fourth dose. When given subcutaneously a non-toxic dose given two or three days later also caused death in rabbits. The experiments in cats have likewise shown unmistakable cumulation. A toxic dose repeated several days later, or even a subminimum dose, if not too small, was fatal when repeated after a few days and four or five small doses administered at intervals of several days were fatal. That this increased susceptibility seems to last for a considerable length of time appears with especial clearness in the guinea pig which received 0.5 cc. oil of chenopodium per kilo in olive oil which failed to cause any symptoms but the same dose was fatal when fed nine days later.

Since the results presented in the present report demonstrate conclusively that chenopodium is a very toxic substance for animals, a word of caution may be addressed to physicians

TABLE II

Cumulative effect of ascaridole and chenopodium

EXP. NO.	WEIGHT	ASCARIDOLE SUBC. INJ.	FIRST DOSE	SECOND DOSE	THIRD DOSE	REMARKS
	grams	*cc. per k.*				
Rabbit 468....	1530	0.22	No symptoms	Fatal		2 doses in 3 days
Rabbit 469....	1075	0.2	No symptoms	Fatal		2 doses in 3 days
Rabbit 473....	840	0.2	No symptoms	Fatal		2 doses in 3 days
Rabbit 479....	790	0.21	No symptoms	No symptoms	Fatal	3 doses in 4 days
		By mouth				
Rabbit 519....	1580	0.3	No symptoms	No symptoms	No symptoms	4 doses in 5 days. 4th fatal
Rabbit 507....	1670	0.3	No symptoms	No symptoms	Fatal	3 doses in 4 days
Rabbit 503....		0.35	No symptoms	Fatal		2 doses in 2 days
		Chenopodium by mouth				
Rabbit 515....	1610	0.6	No symptoms	Fatal in 2 days		2 doses in 3 days
G. P. 196.....	805	0.5				2 doses 9 days apart—duration of life, 1 day.
Cat 278.......	2350	0.3	Slight symptoms	Fatal		Fasting cat—died in less than 20 hours. Chenopodium given in water.
Cat 275.......	2025	0.3	Slight symptoms	Survived		Received 15 cc. olive oil.
Cat 282.......	3075	0.4	Slight symptoms	Fatal		Interval between 1st and 2nd dose 5 days
Cat 280.......	2605	0.2	Slight symptoms	No symptoms	No symptoms	1st, 2d, and 3d doses 3 days apart: 3d and 4th, 2 days. Died after 4th dose
Cat 279.......	2570	0.2	Slight symptoms	No symptoms	No symptoms	Survived 4th dose
Cat 64.......	2410	0.2	Mild symptoms	Died		2d dose 6 days after 1st.

The resistance to ascaridole in these experiments was very unusual. No explanations can be offered at present.

regarding its use in the human subject, more especially as a number of cases of chenopodium poisoning have been reported recently by Levy (17). It is of interest to note that Motter (18) commenting in a recent article in the Public Health Reports on chenopodium poisoning, states that the dose appears to have been excessive and in some cases was repeated, and then quotes several writers who maintained that the use of chenopodium does not cause any important secondary actions.

Since chenopodium is at present being used not only in ascarides but also in hookworm disease which is associated with malnutrition, its exhibition in large and frequently repeated doses cannot be recommended. Brüning (19) and Schuffner and Vervoort (20) recommend as much as one gram every hour until three doses have been given to children from three to thirteen years.

The increased toxicity of chenopodium in starvation and its cumulative effect are important factors as shown in our experiments in determining its toxicity. It is quite possible that the reason that there are so few cases of poisoning in the literature is that castor oil has usually been administered immediately after chenopodium which is quite likely to exert an antidotal influence upon the drug.

SUMMARY AND CONCLUSIONS

Chenopodium produces in cats and dogs, first, symptoms of depression of the higher nerve centers, then convulsions.

The reaction of the rabbit varied, the symptoms being the same as in carnivora in some individuals; in others either excitement or depression only were present.

Guinea pigs presented a picture of poisoning resembling that of rabbits in some respects.

The resistance of dogs, rabbits and guinea pigs to chenopodium was approximately the same. The drug was distinctly more toxic for the cat. The minimum fatal dose, when administered by mouth, was about double that by subcutaneous injection in dogs, cats, and guinea pigs, but the difference was less in the

rabbit. The rate of absorption by subcutaneous injection and by mouth was about the same (as judged by the appearance of symptoms). Ascaridole was about 30 per cent more toxic than chenopodium. Its rearrangement product was less than half as toxic as chenopodium.

The toxicity of chenopodium is distinctly increased in starvation and is decreased by feeding oils and by feeding a rich carbohydrate diet.

Detoxication by glycuronic acid derived from glycogen and glycerides is suggested. The suppression by fixed oils of acute symptoms produced by chenopodium may also be explained by its solubility in oils.

Cumulative effects of ascaridole and of chenopodium were observed in different animals.

BIBLIOGRAPHY

(1) BRÜNING: Zeitschrift für experimental Pathologie und Therapie, **3**, p. 564, 1906.
(2) SALANT: Journal of Pharmacology and Experimental Therapeutics, **2**, p. 391, 1911.
(3) Schimmel and Company, Report, April, 1908.
(4) NELSON: Journal American Chemical Society, **33**, (1911), 1404; **35**, (1913) 84.
(5) WALLACH: Annalen der chemie, **392**, p. 59, 1912.
(6) NELSON: Journal American Chemical Society, **35**, (1913), 86.
(7) HILDEBRANDT: Archiv für experimentelle Pathologie und Pharmakologie, **44**, p. 278, 1900.
(8) MATZEL: Archives internationales de pharmaçodynamie et de therapie, **14**, p. 331, 1905.
(9) NERKING: Münchener Medizinische Wochenschrift, **56**, p. 1475, 1909.
(10) KRAMER: Journal of Experimental Medicine, **17**, p. 206, 1913.
(11) SCHMIEDEBERG AND MEYER: Zeitschrift für Physiologische Chemie, **3**, p. 422, 1879.
(12) HILDEBRANDT: loc. cit.
(13) MATZEL: loc. cit.
(14) MEYER, P:. Zeitschrift für klinische Medizin, **47**, p. 68, 1902.
(15) HILDEBRANDT: loc. cit.
(16) NEUBERG: Ergebnisse der Physiologie, **3**, Part 1, p. 446, 1904.
(17) LEVY: Journal of the American Medical Association, **63**, p. 1946, 1914.
(18) MOTTER: Public Health Reports, **29**, p. 2651, 1914.
(19) SCHÜFFNER AND VERVOORT: Münchener Medizinische Wochenschrift, **60**, 129, 1913.
(20) BRÜNING: Deutsche Medizinische Wochenschrift, **33**, p. 425, 1907.

THE ACTION OF GLANDULAR EXTRACTS ON THE SECRETION OF CEREBROSPINAL FLUID

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Introduction. In our previous communications (1, 2), we have shown that an artificial hydrocephalus can be produced experimentally, either by the injection of aleuronant or by blocking the lower end of the aqueduct of Sylvius with gauze or cotton, while a very rapidly fatal hydrocephalus can be produced by a combination of the two methods. We also demonstrated that the cerebrospinal fluid pressure varied with the pressure in the venous sinuses, and that interference with the carotid circulation has only a transitory effect on cerebrospinal pressure and none on the rate of secretion of the choroid plexus. The absorption of cerebrospinal fluid was shown to be largely through the venous channels, 50 per cent to 60 per cent being absorbed in two hours, while the lymphatic absorption is very slow and small in amount. Neutral phenolsulphonephthalein was found to be the best indicator of the rate of absorption from the subarachnoid space.

In the pursuance of the investigations we have had in mind many clinical problems, e.g., meningitis, brain tumors and hydrocephalus, in which the increase in cerebrospinal fluid plays so important a part in the symptomatology and outcome of the disease. Looking upon the choroid plexus as a secretory organ, and confronted with the failure to deal adequately with the overwhelming increase of cerebrospinal fluid by drainage or other means, the question arose in our minds, can the outflow of cerebrospinal fluid be controlled at its source? In thyroid

extract we have discovered a substance with answers this question affirmatively.

This communication is a continuation of our work on the secretion of the cerebrospinal fluid, the main object of which is the determination of such correlations as may exist between the choroid plexus and other glands. In pursuance of this object we have investigated the influence of saline extracts of various organs on the flow of cerebrospinal fluid.

Comparatively little work has been done on the influence of organ extracts on the choroid plexus although the effects of various salts, drugs and other substances have been demonstrated by several workers.

Action of various substances on the flow of cerebrospinal fluid. Cappelleti (3) in 1900 first demonstrated that both ether and pilocarpine increased the flow while atropine slowed it. Dixon and Halliburton (4) in a recent paper confirmed these findings and also demonstrated that brain extract and an extract of the choroid plexus stimulated the secretion of cerebrospinal fluid. Among the various substances, which they tested for possible effect on cerebrospinal fluid secretion, should be mentioned extracts of choroid plexus and of brain, normal salt solution, Ringer's solution, and concentrated solution of various salts; also urea, inosite, peptone, Liebig's extract, pituitary extract, mussel extract, β-iminazolylethylamine, adrenalin, pilocarpine, atropine, cholesterin, cerebrospinal fluid of other animals, and anesthetics, narcotics and hypnotics. None of these gave noticeable changes in the rate of secretion except brain and choroid plexus extracts. They obtained a very slight increase with cholesterin. Pathological cerebrospinal fluid gave interesting results however. The fluid from cases of general paresis, and in one case of delirium tremens, stimulated the flow, while fluid from patients with other diseases did not show any influence.

Methods. As in previous experiments, dogs under morphine-urethane anesthesia were used. The animals were placed in an inclined position on their backs, with the head flexed; a cannula was inserted into the lower end of the fourth ventricle,

which, in this position, is the most dependent part of the cerebrospinal system. The rate of secretion of cerebrospinal fluid was measured by observing the flow into a graduated glass cannula and each 0.01 cc. advance recorded on the drum. Femoral blood pressure and respiratory tracings were made in the usual way and recorded synchronously with the flow of cerebrospinal fluid. In some of our experiments the sinus pressure was also recorded. This was done through a trephine opening into the torcular herophili; in which was inserted a tight fitting cannula connected with manometer filled with a saturated solution of magnesium sulphate. The saline extracts were injected into the exposed femoral vein.

Saline extracts of the following glands were used: brain, thyroid, pancreas, spleen, kidney, liver, testes, and ovary. The glands were removed from freshly killed dogs and ground with clean sand to a fine paste. Normal saline was added in the proportion of 2 cc. to 1 gram of the fresh gland, except the thyroid and adrenals, where 4 cc. to 1 gram were used. This material was then centrifuged at high speed and the supernatant fluid used for the injections. Human thyroids removed at operation from cases of goitre were treated in the same way.

The action of these extracts was controlled by the injection of other substances such as urine, bile, cerebrospinal fluid, chloroform, ether, amyl-nitrate, magnesium sulphate and physiological saline solution.

Experiments. The following records are typical examples of numerous experiments made with each glandular extract. Glands from several dogs were used in order to eliminate any individual variation which might be present.

SPLENIC EXTRACT

Injections of various amounts of splenic extract gave the following results.

1 cc. splenic extract. Normal rate was 0.0375 cc. per minute (0.05 cc. in 82 sec.) After injection of 1 cc. splenic extract the rate increased to 0.0467 cc. per minute (0.2 cc. in 256 sec.)

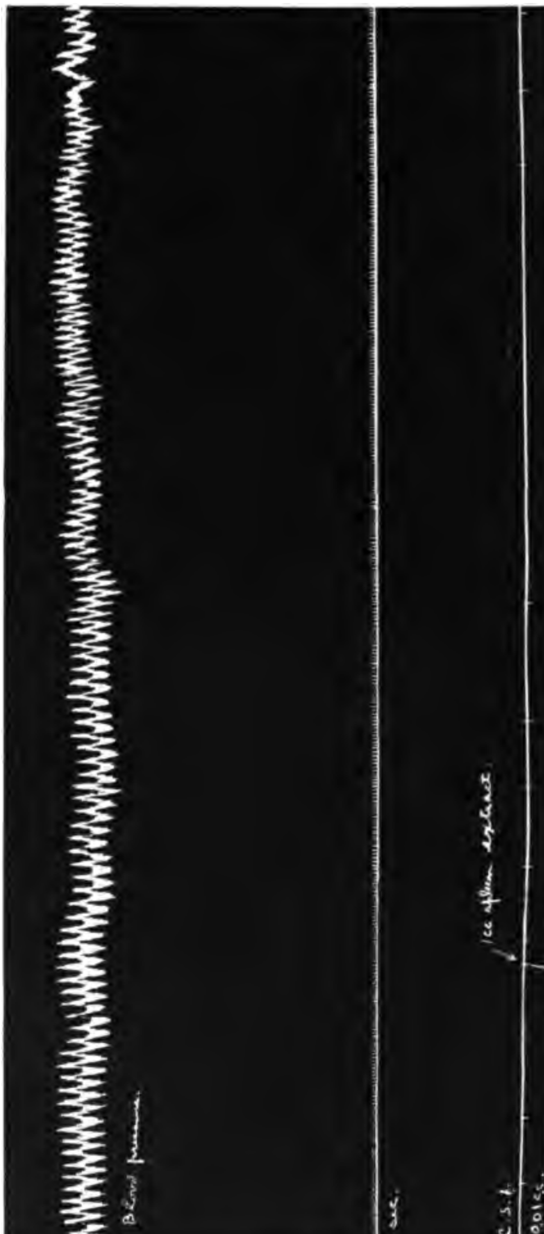

Fig. 1.

or an actual increase of 0.0092 cc. per minute. Very little change in blood pressure resulted. Figure 1.

2 cc. splenic extract. Normal rate was 0.065 cc. per minute (0.13 cc. in 120 sec.) After injection of 2 cc. splenic extract the rate increased to 0.253 cc. per minute (0.43 cc. in 98 sec.) an actual increase in rate of 0.188 cc. per minute. This was followed by an absolute cessation of flow for 197 seconds at which time the flow returned slowly to normal. At the normal rate 0.3195 cc. would have flowed from the cannula during this period. Subtracting this from the actual flow of 0.43 cc. we find there was an actual increase in fluid of 0.1105 cc. above the normal amount for that length of time and an increase in rate of 0.022 cc. per minute. Coincident with the rapid increase in rate a moderate drop in blood pressure was recorded. As the blood pressure began to rise the flow of cerebro-spinal fluid ceased and did not commence until the blood pressure had practically reached normal. The actual rate of flow for the entire period from the time of injection to the return of normal rate is practically normal.

A more marked drop in blood pressure resulted from the injection of the same amount in another dog. In this case the normal rate was 0.0545 cc. per minute (0.06 cc. in 66 sec.). After injection of 2 cc. splenic extract the rate increased to 0.3205 cc. per minute (0.39 cc. in 73 sec.) an increase in rate of 0.266 cc. per minute. A marked drop in blood pressure accompanied this rapid increase in rate. As the blood pressure returned to normal the rate decreased to 0.0162 cc. per minute (0.06 cc. in 222 sec.) a decrease from the normal rate of 0.0383 cc. per minute. The whole period after injection, 295 seconds would yield at the normal rate 0.2679 cc. subtracting this from the actual flow of 0.45 cc. gives an increase in fluid of 0.1821 cc. for the period, and an increase in rate of 0.037 cc. per minute. After the blood pressure had returned to normal, the rate was much slower than before injection so that considering the entire period from the time of injection to the time when a normal rate was resumed we find that practically no actual increase in rate resulted from the injection. Figure 2.

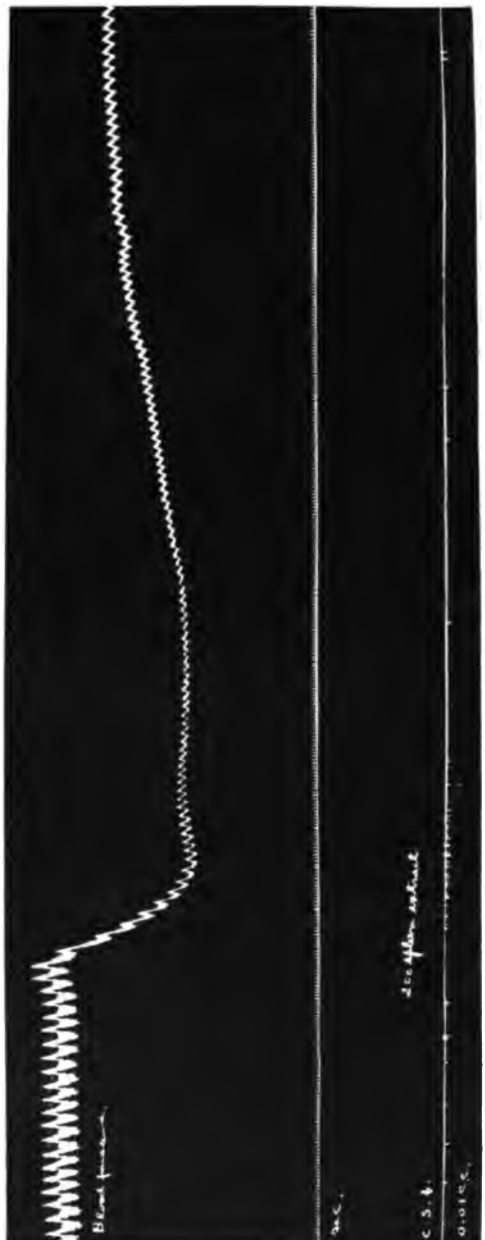

Fig. 2.

4 cc. splenic extract. Normal rate was 0.024 cc. per minute (0.06 cc. in 150 sec.). After injection of 4 cc. splenic extract the rate increased to 0.0986 cc. per minute (0.41 cc. in 249 sec.) an increase in rate of 0.0746 cc. per minute. A steady, though gradual, drop in blood pressure was associated with the increase in rate, which at first was very rapid, but soon decreased to less than normal, so that the final result was the same as before, i.e., practically no increase in rate considering the whole period. Figure 3.

Other splenic injections gave similar results, which may be summarized as a marked drop in blood pressure with a rapid increase in rate of cerebrospinal fluid outflow. The latter was only transitory and was invariably compensated for by the marked decrease in rate or temporary cessation in flow which followed, usually coincident with the gradual return of the blood pressure to normal.

KIDNEY EXTRACT

2 cc. kidney extract. Normal rate was 0.0545 cc. per minute (0.08 cc. in 88 sec.) After injection of 2 cc. kidney extract the rate increased to 0.0634 cc. (0.31 cc. in 293 sec.) per minute, an increase of only 0.0089 cc. per minute. Immediately after injection an irregularity marked by a slight rise and fall in the blood pressure was recorded. This was coincident with the rapid and very transitory increase in rate which was compensated for by a marked decrease in rate immediately following so that the average rate for the whole period is very close to the normal.

2 cc. kidney extract. On this dog the effect of 2 cc. of extract was much more marked than in the previous animal. Normal rate was 0.0818 cc. per minute (0.06 cc. in 44 sec.) after injection the rate increased to 0.415 cc. per minute (1.19 cc. in 172 sec.) an increase in rate of 0.3697 cc. per minute, or over five times the normal rate. Coincident with the rapid outflow was the marked drop in blood pressure. The rate of flow then decreased to 0.066 cc. per minute (0.07 cc. in 63 sec.) and remained slower than normal until the blood pressure returned to its

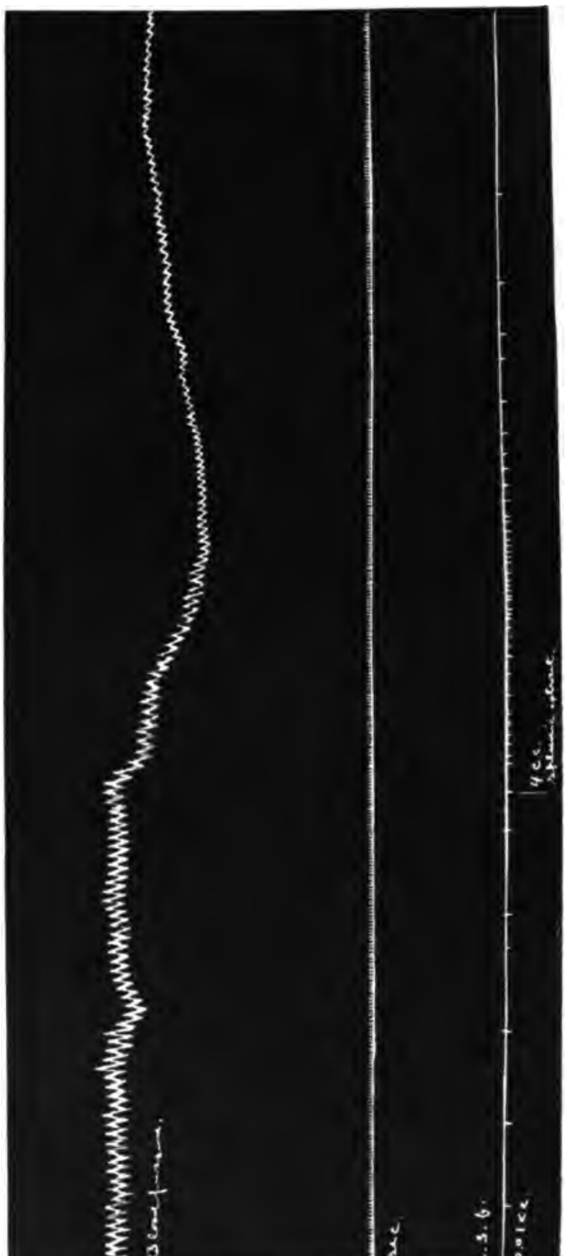

Fig. 3.

former level. The actual rate for the entire period was not increased. Figure 4.

4 cc. kidney extract. Although twice the amount given in the previous experiment was used in this dog the effect was not as pronounced. Normal rate was 0.0499 cc. per minute (0.03 cc. in 44 sec.). After injection of 4 cc. kidney extract a sudden slight drop in blood pressure was recorded and the rate of the cerebrospinal fluid was increased to 0.056 cc. per minute (0.28 cc. in 300 sec.) an increase of 0.0151 cc. per minute. The slight drop in blood pressure was very transitory as it was followed by a rapid return to normal, during which time the flow dropped slightly below the normal rate.

This experiment in connection with those recorded above demonstrates that the amount of kidney extract injected does not directly influence the rate of flow of cerebrospinal fluid, but only has a transitory effect through the fall in blood pressure.

PANCREATIC EXTRACT

The saline extract of pancreas, always gave a marked drop in blood pressure even when comparatively small amounts were injected.

2 cc. pancreatic extract. Normal rate was 0.256 cc. per minute (0.01 cc. in 106 sec.). With the sudden drop in blood pressure the rate increased to 0.56 cc. per minute (0.45 cc. in 50 sec.) an increase of 0.504 cc. per minute. The flow ceased for the next 12 seconds, while in the succeeding 169 seconds 0.2 cc. was drawn back into the cannula. A period of rest of 86 seconds followed before the fluid started flowing at the normal rate. The sucking back of the fluid was probably due to collapse of the cerebral sinuses and was coincident with the gradual rising blood pressure. The very rapid outflow coincident with the drop in blood pressure was probably due to the sudden dilatation of the cerebral sinuses forcing out fluid which had accumulated in the ventricles and possibly the cisterna magna. If we compute the rate of flow for the entire period after injection (0.26 cc. in 317 sec.) we find the rate would be

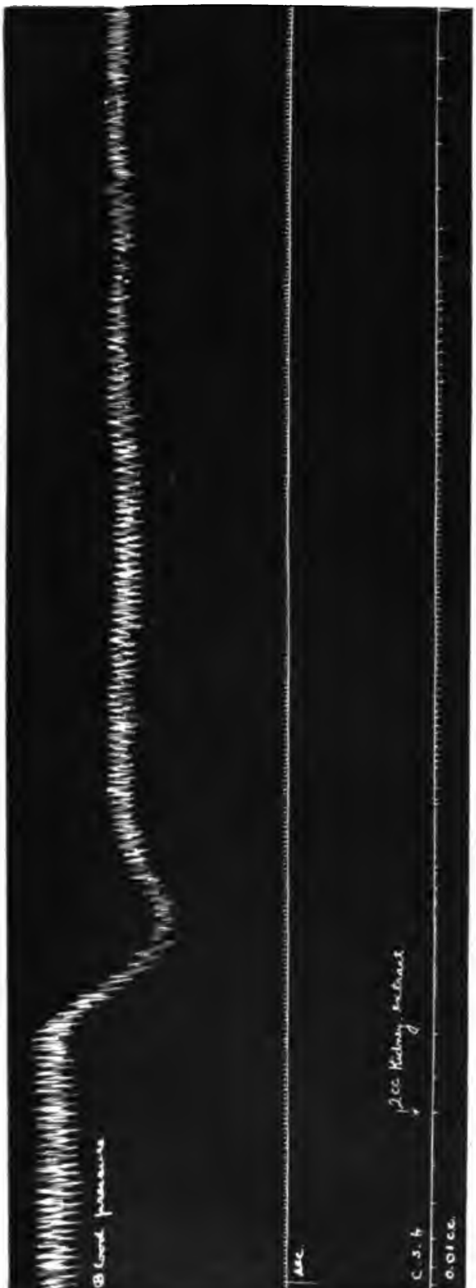
Blood pressure
sec
C.S.F.
0.01 c.c.
2 cc Kidney extract

Fig. 4.


Blood pressure
sec
C.S.F.
0.01cc

Fig. 5.

0.0492 cc. per minute or a decrease from the normal rate of 0.068 cc. per minute. It is thus seen that while a marked increase in rate of secretion of cerebrospinal fluid followed the injection, this apparent increase was simply the forcing out of fluid already in the cavities of the cranium, by the distention of the sinuses resulting from the sudden fall in blood pressure. The actual rate of secretion was not increased. Figure 5.

A second dog injected with the same amount gave similar results. Normal rate was 0.0671 cc. per minute (0.03 cc. in 134 sec.). After injection of 2 cc. pancreatic extract a sudden drop in blood pressure with rapid rise to normal was recorded. A rapid outflow followed the drop, 0.45 cc. flowing in 78 seconds (0.346 cc. per minute). This was followed by cessation of the flow for the next 138 seconds. The computed rate of cerebrospinal fluid flow for the entire period after injection gives an average rate of 0.127 cc. per minute, an increase of 0.06 cc. per minute or twice the normal rate. The flow was slower than normal for the succeeding three minutes which brings down the average rate per minute for the period after injection to practically normal.

TESTICULAR SECRETION

The normal rate was 0.0574 cc. per minute (0.09 cc. in 94 sec.). After injection of 2 cc. testicular extract there was a slight irregularity in the blood pressure curve and a slight increase in the rate of cerebrospinal fluid flow. This was followed by a slightly slower rate. The rate for the entire period after injection was 0.0636 cc. per minute (0.3 cc. in 283 sec.) an increase over the normal rate of only 0.0062 cc. per minute which is easily within the normal variation in rate. Figure 6.

OVARIAN EXTRACT

Since the ovary consists of such a large amount of connective tissue in proportion to its glandular constituents larger amounts of this extract were used.

Normal rate was 0.0277 cc. per minute (0.05 cc. in 108 sec.). After injection of 5 cc. ovarian extract the rate increased to 0.0391

Fig. 6.

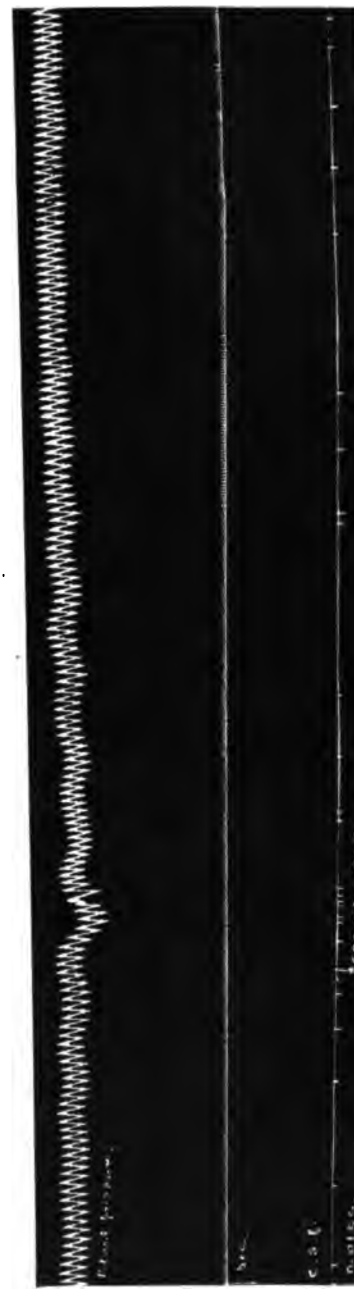

Fig. 7.

cc. per minute (0.22 cc. in 322 sec.) an increase of 0.0114 cc. per minute. This slight increase in rate was due to a sudden short increase in rate coincident with a slight drop in blood pressure. The flow following this period was slower than normal. Figure 7.

LIVER EXTRACT

Comparatively small amounts of this extract cause marked sudden falls in blood pressure, resembling pancreas in this regard.

Normal rate was 0.057 cc. per minute (0.06 cc. in 63 sec.). After injection of 3 cc. liver extract, a sudden, almost vertical drop in blood pressure was recorded and the rate of outflow increased to 0.437 cc. per minute (0.21 cc. in 29 sec.). The blood pressure quickly returned to normal and the rate of outflow dropped to only 0.0108 cc. per minute (0.05 cc. in 276 sec.). The actual rate for the entire period after injection is 0.0509 cc. per minute, which is slightly less than the normal, but within the normal limits of variation. Figure 8.

The experiment with liver extract again demonstrates that while a sudden increase in the rate follows an injection, it is not due to an increase in rate of secretion of the choroid plexus, but is the result of the sudden drop in arterial blood pressure affecting the cerebral sinuses.

BRAIN EXTRACT

5 cc. extract. The normal rate of cerebrospinal fluid flow before injection was 0.0428 cc. per minute (0.09 cc. in 120 sec.). After injection of 5 cc. brain extract the flow increased in rate to 0.286 cc. per minute (0.13 cc. in 90 sec.) and then slowed down to 0.0172 cc. per minute (0.05 cc. in 174 sec.). A slight drop in blood pressure was recorded during which the rate of flow was increased 0.2483 cc. per minute over normal. The actual increase in rate for the entire period after injection was 0.109 cc. per minute (0.48 cc. in 264 sec.). Figure 9.

2 cc. extract. Normal rate before injection was 0.0955 cc. per minute (0.18 cc. in 113 sec.). After injection of 2 cc. brain


Blood pressure
5 cc liver extract

Fig. 8.

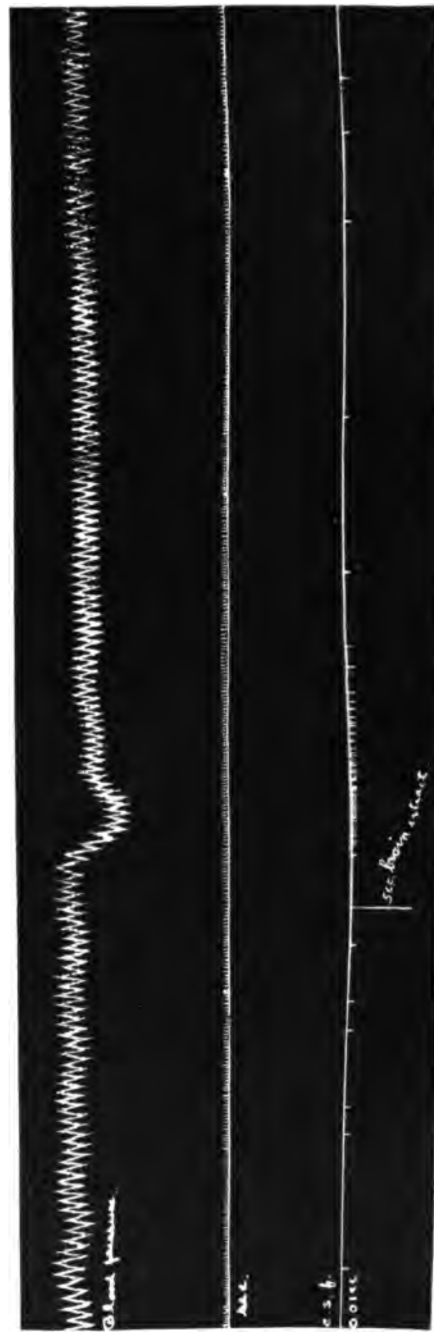
Blood pressure
5 cc brain extract

Fig. 9.

extract the rate increased to 0.207 cc. per minute (0.53 cc. in 153 sec.) then ceased for 152 sec. A very slight rise in blood pressure followed by a slight fall occurred. The actual increase in rate for the entire period after injection was 0.104 cc. per minute.

2 cc. extract. Normal rate was 0.0128 cc. per minute (0.08 cc. in 112 sec.). After injection of 2 cc. brain extract a slight fall in blood pressure occurred and the rate of flow of cerebrospinal fluid was increased to 0.135 cc. per minute (0.14 cc. in 209 sec.) or an actual increase in rate of 0.0922 cc. per minute. Figure 10.

5 cc. extract. Normal rate 0.066 cc. per minute (0.07 cc. in 63 sec.) After injection of 5 cc. brain extract a sudden drop in blood pressure was recorded together with a marked increase in rate of cerebrospinal fluid flow. The rate rose to 0.297 cc. per minute (0.42 cc. in 85 sec.). This was followed by the retraction of 0.02 cc. of fluid and a complete cessation in flow for 195 sec. The computed rate for the entire period after injection is 0.0857 cc. per minute an increase of 0.0191 cc. per minute over the normal rate. In this case the marked fall in blood pressure due to the large amount of extract injected apparently has decreased the actual increase in rate which smaller injections invariably show. This cannot be compared with other glandular extracts such as pancreas, liver or spleen, for with these extracts an amount small enough to have very little effect on blood pressure does not increase or decrease even the transitory rate of flow.

The experiments with brain extract demonstrate that there is an actual increase in rate of flow of cerebrospinal fluid independent of the amount of fall in blood pressure. With the injection of the other extracts previously recorded a marked outflow was registered with each drop in blood pressure, but this was invariably followed by a marked slowing or cessation in the outflow or even an actual withdrawal of the fluid back into the cranial cavity. When the rate of flow was computed for the entire period after injection, i.e., the period of rapid outflow plus the period of cessation, the rate was shown to be practically normal, the increase being only an apparent one.

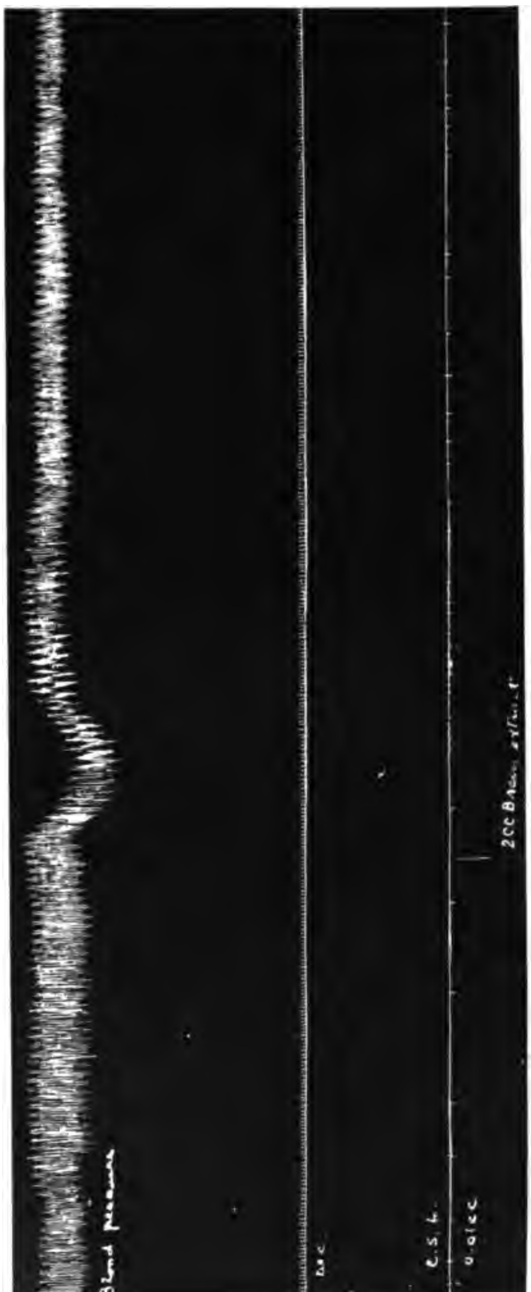

Fig. 10.

THYROID EXTRACT

In our first experiments with thyroid extract we found that the rate of secretion was markedly reduced, therefore the records after injection of thyroid extract were continued over much longer periods (sometimes five or six hours).

5 cc. human thyroid extract. Normal rate was 0.0335 cc. per minute (0.61 cc. in 18 min. 12 sec.). Immediately after injection of 5 cc. thyroid extract there was a moderate drop in blood pressure which persisted for 5 minutes. The rate increased to 0.0714 cc. per minute (0.27 cc. in 3 min. 46 sec.). Following the transitory increase in rate it markedly decreased and after 6 minutes 0.05 cc. of fluid was drawn back into the cannula. The results after injection were computed for 30-minute periods. The rate for the first period was 0.00933 cc. per minute; for the second, 0.00233 cc. per minute; for the third, 0.0103 cc. per minute; for the fourth, 0.0136 cc. per minute; for the fifth 0.0115 cc. per minute. The rate of flow for the whole period after injection, two and a half hours, was 0.0101 cc. per minute, a decrease from the normal rate of 0.0234 cc. per minute. It is thus seen that the normal rate was over three times as rapid as the rate after injection of only 5 cc. thyroid extract.

5 cc. of a 2 per cent. extract of desiccated beef thyroid (commercial). The normal rate of cerebrospinal fluid flow before injection was 0.065 cc. per minute. After injection the rate was slightly increased coincidently with a very slight drop in blood pressure. The results after injection were computed for 30 minute periods as before. The rate for the first period was 0.031 cc. per minute; for the second, 0.0193 cc. per minute; for the third, 0.0153 cc. per minute; for the fourth, 0.0413 cc. per minute; for the fifth 0.023 cc. per minute; for the sixth, 0.0223 cc. per minute; for the seventh, 0.0123 cc. per minute; for the eighth, 0.011 cc. per minute. This gives an average rate for the four hours after injection of 0.0219 cc. per minute. This is a decrease from the normal rate of 0.0431 cc. per minute. As in the previous experiment, the normal rate of secretion of cerebrospinal fluid is three times as fast as after injection of

thyroid extract. It is evident from this experiment that the commercial desiccated thyroid is in every way as efficient in decreasing the secretion of the choroid plexus as is the fresh gland.

3 cc. extract of dog's thyroid. The normal rate of cerebrospinal fluid flow for 30 minutes was 0.04 cc. per minute. After injection of 3 cc. thyroid extract a slight transitory drop in blood pressure was recorded together with a slight temporary increase in the flow of cerebrospinal fluid. The results after injection were computed for 30 minute periods. The rate for the first period was 0.0156 cc. per minute; for the second, 0.0233 cc. per minute; for the third 0.0233 cc. per minute; for the fourth, 0.019 cc. per minute; for the fifth 0.0146 cc. per minute. The average rate for the two and a half hours after injection was 0.0191 cc. per minute. A decrease from the normal rate of 0.021 cc. per minute. At the end of the first 10 minutes in the fourth period 3 cc. of a filtered extract of human goitre was injected without any change in blood pressure or immediate change in rate of secretion, although the rate for the fourth and fifth periods are slightly slower than that for the second and third which may be the result of the second injection.

Physiological saline solution was injected as a control of the saline glandular extracts. The normal rate of flow was 0.026 cc. per minute (0.07 cc. in 161 sec.). After injection of 5 cc. normal saline the rate was 0.026 cc. per minute (0.07 cc. in 160 sec.), exactly the same as before injection. No change in the blood pressure was recorded.

Amyl-nitrate produces results similar in every way to those obtained with organ extracts. In one typical experiment the normal rate of outflow was 0.065 cc. per minute. After inhalation of 5 min. of amyl-nitrate the blood pressure dropped suddenly. The rate of outflow was increased to 0.0341 cc. per minute (0.54 cc. in 95 sec.). This period was followed by a complete cessation of the flow with retraction of 0.09 cc. of fluid as the blood pressure began to rise. The flow then remained stationary for several minutes. The actual flow from the time of injection to time when the normal rate was resumed practically equals the computed normal rate for this period. It is

therefore apparent that the sudden increase in rate was directly associated with the drop in blood pressure as in organ extracts and that no actual increase in secretion resulted.

Similar results were obtained with urine, bile and magnesium sulphate.

SUMMARY AND DISCUSSION

The experiments just submitted illustrate the influence on the rate of outflow of cerebrospinal fluid after the intravenous injection of saline extracts of spleen, kidney, pancreas, testes, ovary, liver, brain and thyroid. A cursory examination of these findings gives the impression of a mass of unrelated, even contradictory facts, *but closer study shows that there are certain phenomena which are common to all these organ extracts, i.e., a more or less marked drop in blood pressure immediately after each injection and a corresponding increase in the rate of cerebrospinal fluid outflow. This depressor effect on the arterial blood pressure is a well known action of nearly all glandular extracts.* The work of Pearce (5), Vincent (6) and many others has demonstrated the constancy of this action and manner of its production. In our work we wish to show the relation which exists between the blood pressure changes and the rate of cerebrospinal fluid outflow after the injection of glandular extracts.

The coincident rapid increase in rate of outflow of cerebrospinal fluid which is invariably associated with this drop in arterial blood pressure has been observed by Dixon and Halliburton (4) and with one glandular extract by Weed and Cushing (7). The former consider the cerebrospinal fluid pressure to be independent of vasomotor changes and therefore conclude that the sudden increase in rate is the result of a hypersecretion of the choroid plexus due to the partial asphyxiation of the secretory cells. Weed on the other hand inclines to the view that the haemodynamic response after injection may account in some cases for the rapid increase in rate.

Our own opinion, which was briefly stated in the Chairman's address before the Section on Surgery of the American Medical Association, June, 1914, *is that the rapid rate of outflow after*

injection of organ extracts is directly due to the drop in blood pressure and not to asphyxiation of the choroid plexus. Our opinion is based upon two distinct but correlated observations. First, a study of the arterial and cerebral sinus pressure before and after the injection. Secondly, a study of the rate of outflow of the cerebrospinal fluid before and after injection.

The haemodynamic influence was studied by tracings taken from the femoral blood pressure together with the record of the coincident changes in the venous pressure in the cerebral sinuses. The latter was obtained by means of a magnesium sulphate manometer connected with a cannula inserted in the torcular herophili. The blood pressure changes were controlled by respiratory tracings, but in our present discussion the latter can be disregarded as under the morphin-urethane anesthesia the respiratory changes are practically negative.

The administration of any depressor substance such as splenic extract, ether, amyl-nitrate, or magnesium sulphate, caused a marked drop in arterial blood pressure followed by a slow rise to normal. Practically coincident with the drop in arterial blood pressure was a sudden rise in the cerebral sinus pressure. This usually occurred immediately after the sudden drop and not with it. The sinus pressure continued to rise as long as the arterial blood pressure remained at its lower level. As the femoral pressure gradually returned to normal the sinus pressure slowly dropped. The outflow of cerebrospinal fluid followed the latter very closely. With each rise of one mm. in the sinus pressure 0.01 cc. or more of cerebrospinal fluid flowed into the graduated cannula. As soon as the sinus pressure dropped the flow of the fluid in the cannula ceased and frequently was drawn back into the ventricles. This demonstrates that the rate of outflow of the cerebrospinal fluid is intimately connected with sudden changes of pressure in the cerebral venous sinuses.

The element of asphyxiation can be ruled out as no change in the respirations occurred. There is no reason to suppose that the choroid plexus is more sensitive to asphyxiation than the respiratory center which certainly would have responded

with increased respiratory movements if even a slight degree of asphyxiation had resulted.

There is one possible complication which should be noted at this time. When ether anesthesia is pushed to the danger point the cerebral sinus pressure rises very high, in fact with a normal of 128 mm. of saturated magnesium sulphate it may rise to 190 or 200 mm. At this time the flow of cerebrospinal fluid is very rapid and more than equals the amount which the ventricles could probably hold. In this case the only explanation which seems feasible is that the very high venous pressure must cause some transudation of fluid through the venous walls. The hypothesis that this enormous increase of fluid is due directly to ether stimulation of the choroid plexus is hardly tenable, since in smaller doses, which do not raise the venous pressure, very little stimulation occurs, certainly nothing which could be compared with the relative size of the dose in the first instance. The possible augmentation of the choroid plexus secretion by the addition of a transudate does not materially affect our former findings, as after the injection of organ extracts no such enormous increase in sinus pressure occurs.

Further proof that the rate of outflow is influenced by the sinus pressure is shown by the reaction to small amounts of depressor substances. An injection of splenic extract, which is sufficiently small not to affect the blood pressure does not cause an increase in the rate of outflow of cerebrospinal fluid.

We are thus led to the conclusion that the sudden increase in rate of outflow following the injection of organ extracts is the result of sinus distension which forces out fluid already present in the ventricles and cisterna.

The second series of observations, which substantiate the opinion that circulatory disturbances are the direct cause of the increased rate of cerebrospinal fluid flow after injections of organ extracts, deal entirely with the rate of outflow and will be considered entirely apart from vasomotor conditions.

The injection of saline extracts of spleen, kidney, pancreas, testes, ovary and liver always results in a temporarily increased rate of cerebrospinal fluid outflow. If the increase has been marked the flow following this transitory increase usually ceases.

A very marked expulsion of fluid is almost invariably followed by retraction of the fluid into the cisterna or ventricles. The comparatively slight increase after ovarian or testicular extract is usually followed by a simple slowing of the rate below normal. The actual rate after injection should therefore be the rate from time of injection to the time when the normal rate was resumed, or at least until the arterial and venous pressure had returned to normal. The rate for the entire period after injection of these five organs was shown to be practically the same as the normal rate before injection. This must of course take into consideration the amount of fluid retracted after the flow had ceased.

Irrespective of the reaction, i.e., whether the flow became subnormal, whether it ceased entirely, or whether it was drawn back into the cranial cavity, no noteworthy deviations from this rule were found.

It was necessary in order to study the flow after the temporary increase in rate to make much longer tracings than are usually made. For this reason we discontinued the use of the smoked paper as shown in our figures and substituted long rolls of paper on which the tracings were made with ink. In this way records of five or six hours could be made without changing the apparatus in any way. We are inclined to think that some of the findings of Dixon and Halliburton are the result of observations over too short periods.

The injection of brain extract gave the same drop in blood pressure and sudden increase in cerebrospinal fluid outflow which was noted with the other glandular extracts. It differs however, from the previous extracts in that the rate for the entire period is greater than normal. In other words brain extract gives an actual increase in the outflow of cerebrospinal fluid. This was determined by subtracting the normal rate from the rate for the entire period after injection (from time of injection to return of normal rate). The average increase in rate of the four experiments quoted is 0.106 cc. per minute.

Thyroid extract gives a decrease in the rate of cerebrospinal fluid outflow, an opposite effect to that of brain extract. With doses of 3 cc. to 5 cc. of thyroid extract the usual drop in blood

pressure with the increased rate of flow of cerebrospinal fluid always occurs. But following this increased rate with its compensatory slower rate or cessation of flow is a prolonged period of markedly decreased rate. This result is obtained by the injection of saline extracts of fresh dog thyroid, fresh human thyroid (colloid goitre), fresh rabbit thyroid and commercial desiccated beef thyroid. The extracts of dog thyroid reduced the flow over one-half during a period of two and a half hours, while extract of human and dessiccated beef thyroid reduced the rate to one-third of the normal, during a period of two and a half hours respectively. Rabbit thyroid injected in doses corresponding to the saline extract of two thyroids (from one rabbit) for a 10 kilo dog gave similar results without the fall in blood pressure, and the resultant increase in cerebrospinal fluid outflow. *In this experiment the decrease in rate appeared almost immediately after the injection and lasted for four and one-half hours at which time the dog was killed.* In similar experiments much longer records have been made, some over a period of five and six hours during which the rate remained much slower than normal. Control experiments have demonstrated that with the methods we used the cerebrospinal fluid will flow at nearly a uniform rate for this length of time.

Further corroborative evidence as to the specific action of thyroid extract on the choroid plexus is furnished by the first clinical case of hydrocephalus in which we tried the effect of feeding dessiccated thyroid. This baby developed hydrocephalus when three weeks old. The thyroid feeding was commenced one month later, at which time the hydrocephalus was marked. Four weeks later the child showed improvement and now after six months has increased one-third in weight, grown four inches taller, and shows no signs of hydrocephalus.

Recently we have carried on a series of experiments with diiodotyrosin which was kindly furnished us by Dr. Treat B. Johnson, who prepared the material. This synthetic substance apparently has an effect similar to thyroid extract although not as marked either in action or duration. A full report this substance will appear in a later paper.

CONCLUSIONS

From the experiments here reported we can state:

(1) Saline extracts of pancreas, spleen, kidney, liver, ovary and testes do not influence the rate of secretion of the choroid plexus.

(2) The apparent increased rate after the injection of extracts of these glands is a mechanical rather than a secretory effect.

(3) This mechanical effect is directly due to the fall in arterial blood pressure which increases the pressure in the cranial venous sinuses thus forcing out the preformed fluid in the ventricles and cisterna magna.

(4) Brain extract causes an increase in secretion independent of blood pressure changes.

(5) Thyroid extract, either from fresh glands or the commercial desiccated beef preparation is the only glandular substance which has a specific inhibitory effect on the secretory activity of the choroid plexus, quite independently of blood pressure changes.

BIBLIOGRAPHY

(1) Frazier, C. H. The Cerebrospinal Fluid as a Problem in Intracranial Surgery. Jour. Amer. Med. Assoc., 1914, lxiii, p. 287.

(2) Frazier, C. H. and Peet, M. M. Factors of Influence in the Origin and Circulation of the Cerebrospinal Fluid. Am. Jour. of Physiol., vol. 35, 1914, p. 268.

(3) Capelletti, L: L'efflusso del liquido cerebro-spinale dalla fistola cefalorachidiana in condizioni normali e sotto l' influenza di alcuni farmaci. Atti dell' Accad. d. Science Med. e Nat. d. Ferrara, 1900, lxxiv, 85.

(4) Dixon, W. E. and Halliburton, W. D. The Cerebro-spinal Fluid. I. Secrĕtion of the Fluid. Jour. of Physiol., 1913, vol. xlvii, p. 215.

(5) Pearce, R. M: An Experimental Study of the Influence of Kidney Extracts and of the Serum of Animals with Renal Lesions upon the Blood Pressure. Jour. of Exp. Med., 1909, vol. xi, p. 430.

(6) Vincent, S. and Sheen, W: The Effect of Intravascular Injections of Extracts of Animal Tissues. Jour. of Physiol., 1903, xxix, p. 242.

(7) Weed, Lewis H. and Cushing, Harvey: The Effect of Pituitary Extract upon its Secretion (Choroidorrhoea). Jour. of Physiol., 1915, vol. xxxvi, p. 77.

SOME METABOLIC INFLUENCES OF BATHING IN THE GREAT SALT LAKE

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The value of regular and systematic bathing as a health-producing agency is most emphasized by those who make periodical visits to bathing resorts and by the physicians under whose direction they live while there. An explanation for the salutary effect of the water is usually that it "stimulates metabolism." Whatever influence the bathing may have, the effects of simple food, regularity, new surroundings, and the careful attention of physicians to all ailments, small and large, are factors, which, according to Umber (1) and others (2) are more favorable, therapeutically, than any constituents of the water. The desirable results of the use of large amounts of water internally, which is usually a part of the régime, also come into consideration (3) (4).

According to Matthes (5) in v. Noorden's treatise on metabolism the effects of baths and bathing may be credited either to the temperature of the water or to its mechanical or chemical action.

With respect to the influence of temperature, both cold and hot baths may bring about an increased metabolic exchange, the combustion of non-nitrogenous substances being first affected. If material disturbance in the heat balance occurs, protein metabolism may also appear to be influenced. Little is known regarding the individual nitrogen components. Formanek (6) investigated the influence of cold baths on total nitrogen and uric acid excretion in a human subject on a uniform mixed diet and found an increase in both, with an increased fecal nitrogen

at the same time, indicating poorer utilization. In similar experiments on a fasting dog (7) in which the body temperature fell to 29.3° the nitrogen excretion rose from 1.4 to 4.6 grams per day and remained at this high level for several days. In a hot bath Rubner (8) found metabolism stimulated, as measured by respiratory exchange. If the heat is sufficient to raise the body temperature considerably, there is an increased protein metabolism, not, according to v. Noorden, as a primary result of increased body heat, but after increased carbohydrate metabolism has depleted the glycogen stores. A possible direct influence can hardly be excluded. A stimulation of protein metabolism as the result of hot baths has been observed by several workers (9) (10) (11).

The metabolic influences of different substances in the bathing water has been looked for chiefly in brine baths of various concentrations, in sea water baths, in sool baths (Stassfurt salt) with and without carbon dioxide, in mustard baths, in those containing radium emanation, and in many others.

(a) *Protein metabolism.* The work of earlier investigators is conflicting, indicating in some cases an increased, in others a decreased, nitrogen excretion following brine and sool baths. Bahrmann and Kochmann (2) question these results because the preliminary periods were generally too short to observe nitrogen metabolism properly. Also the nitrogen in feces and sweat is not accounted for. They conclude that the effect of sool baths on metabolism is no different from that of ordinary baths at the same temperature. In experiments on two strumous children, five and eight years old, Heubner (12) found increased nitrogen excretion during a bathing period in which the baths, beginning with pure warm water, were next 3 per cent and then 5 to 7 per cent NaCl. The high nitrogen excretion continued through the final period. The calorific value of the diets was the same, and the nitrogen content almost identical, yet the increased nitrogen elimination produced a minus balance in the less well nourished of the two children throughout the latter part of the bathing period and the final period.

(b) *Metabolism of sodium chloride.* Regarding variations in

sodium chloride excretion the findings are also contradictory, and Bahrmann and Kochmann (2) are unwilling to use most of the data because in some cases the analytical methods were not the best, and because the laws of sodium chloride metabolism are not well enough understood. That variations in the amount of salt excreted are at least not to be explained by absorption of salt through the skin is generally accepted. Heubner (12) found a retention of NaCl in the poorly nourished subject during the bathing period, while in the well-nourished the NaCl elimination was uniform throughout.

That the salts of a bath may remain on the skin a considerable time after the bath if not rinsed off with fresh water was shown by Lehmann (13) who was able to demonstrate spectroscopically the presence of lithium and strontium on the skin a week after a bath containing these salts, which may therefore continue to exercise an effect on the skin (14), if, indeed, they have an effect. Several ingenious suggestions are made, that the salt on the skin makes a cloak, lessening the heat loss through evaporation (15), or that the water taken up by the hygroscopic salts stimulates the skin and increases oxidation (16).

(c) *Gaseous metabolism.* Here again the early observations are contradictory, but the opinion of Winternitz (17) that sool baths, even 15 per cent salt, have no other effect than sweet water baths of the same temperature is no longer questioned. In the extensive work on the influence of sea climate and bathing on man Loewy and his co-workers (18) found an increased oxygen consumption. During the first few days a decrease in respiratory quotient was noted. Incidentally also, they found nitrogen metabolism increased in one individual, decreased in another, and during the bathing period a sodium chloride excretion greater than the ingestion. The effect of a sea bath, they state, is entirely different from that of a cold tub, and the unknown factors in this explanation probably account for the many conflicting results.

The Great Salt Lake offers an unusual salt concentration. Because of this and because of the beneficial influences that are claimed for its water, it seemed desirable to learn whether bath-

ing in this water modified metabolic processes in any tangible way.[1]

Two subjects were maintained on a uniform diet as follows:

	Subject 1	*Subject 2*
Graham crackers, per meal	140.0 g.	110.0 g.
Peanut butter, per meal	20.0	20.0
Butter, per meal	25.0	25.0
Milk, per meal	425.0 cc.	400.0 cc.
Water, per day	1200.0 cc.	600.0 cc.
Tea (evening), per day		300.0 cc.
Nitrogen per day	14.0 g.	12.6 g.
Calories per day	3380.0	2962.0

The experiment was divided into five periods: (1) a preliminary period of five days, (2) a bathing period of four days, (3) two days without bathing, (4) four days of bathing, (5) a final period of two days. Periods 2, 3 and 4 are sometimes referred to as the bathing period. The daily routine of the subjects was practically uniform throughout the experiment. During the bathing days the laboratory work was augmented by a 15-mile ride to Saltair and back, and by the usual exercise while in the water. No definite activity was substituted for this during periods 1, 3 and 5.

The composition of the water of Great Salt Lake has been the subject of several inquiries (19), and has been found to vary considerably with the seasons and with the amount of rainfall. Generally speaking the water shows a minimum of total solids after the spring thaws, the amount increasing through the dry summer months and coming to a maximum in the fall before the winter precipitation begins. Analysis of a sample taken during the bathing showed a specific gravity of 1.152, 19.17 per cent total solids, of which 93.34 per cent were chlorides calculated as NaCl. After the bath the face and hands were rinsed with fresh water, the rest of the body being wiped immediately with a dry towel. The original plan had been to make the bath during period 2 from twenty to thirty minutes long, and during period 4 from forty-five to sixty minutes. Ordinarily the weather in Salt Lake is warm enough, by the end of June, to make this possible, but a week of cold,

[1] It is a pleasure to acknowledge the assistance of Saltair Beach Company, who provided transportation and the privileges of their bathing equipment.

rainy weather at the end of the experiment made it quite impossible to do this and still have anything like normal bathing. The local weather bureau records a higher precipitation (3.37) for the month of June 1913, than has occurred in any June since the local weather bureau was established in 1874. The excess over the normal was 2.6, and practically all of this precipitation came during the days of the second bathing period.

The urine was collected in 24-hour samples, preserved by thymol, and cold, and analyzed in duplicate for total nitrogen (Kjeldahl), creatinine (Folin), chlorides (Dehn-Clark) and uric acid (Folin-Macallum).

Acidity (Folin) was determined during the latter part of the experiment, but the results are not significant; ammonia was determined throughout, but the discovery, later, that the distilled water was untrustworthy caused us to question these results, even though duplicate analyses were obtained. This also necessitated a repetition of the total nitrogen determinations. The original plan of the work had included the determination of fecal nitrogen and chlorides, and of urinary indican, and observations of blood pressure and blood count as affected by the bath, but these had to be relinquished before any conclusive results were obtained because of the inadequacy of the working force. The omission of blood pressure determinations is particularly unfortunate because, in view of certain clinical results, the pressure of the heavy water on the exterior of the body may materially increase blood pressure, and as a result, perhaps modify metabolic processes in the tissues.

The results of the urine analysis, and a record of the length and temperature of the baths are shown in Table 1. Figure 1 shows graphically the variations in the urine constituents during the experiment.

Total nitrogen. The results indicate a slightly increased nitrogen elimination during the bathing period, 7.16 per cent above the average of the non-bathing days in Subject 1, where the variations follow the periods rather closely. In Subject 2 the effect is not as clear, though periods 2 and 4 together average 5 per cent greater nitrogen excretion than the average for all non-bathing periods. The fact that nitrogen elimination in

period 3 (two days without bathing) is higher than in period 2 and almost as high as in period 4 may be due to a lag which is noted in much of the data for Subject 2. The fall in body temperature during and following the baths (p. 000) was small and

TABLE I.

Subject 1

DAY OF EXPERIMENT	DATE	BODY WT.	URINE VOLUME	TOTAL N	URIC ACID	CREATININE	CHLORIDES	TEMP. OF AIR	TEMP. OF WATER	LENGTH OF BATH MINUTES
		Pounds	*c.c.*	*Grams*	*Grams*	*Grams*	*Grams*	*Cent.*	*Cent.*	
1	6/13	131.4	1722	11.72	0.38	1.24	5.87			
2	14	131.5	805	10.67	0.36	1.16	4.80			
3	15	131.6	1130	11.84	0.37	1.02	5.44			
4	16	131.8	1030	12.04	0.33	1.28	6.27			
5	17	132.0	1725	12.15	0.28	1.24	6.70			
Average.				**11.68**	**0.34**	**1.19**	**5.82**			
6	18	132.5	1558	11.98	0.31	1.19	7.91	26.0	22.2	15
7	19	132.3	1265	11.84	0.33	1.28	7.76	31.0	23.6	30
8	20	131.7	1448	12.35	0.29	1.26	7.05	23.5	23.0	20
9	21	131.9	1162	12.21	0.32	1.16	6.01	27.0	24.5	30
Average.				**12.10**	**0.31**	**1.22**	**7.18**			
10	22	132.3	870	11.22	0.23	1.13	5.64			
11	23	132.7	1650	12.64	0.31	1.24	7.15			
Average.				**11.93**	**0.27**	**1.19**	**6.40**			
12	24	133.3	2110	14.50	0.25?	1.21	7.91	21.8	22.8	45
13	25	133.3	1860	13.16	0.26	1.31	8.84	20.5	22.5	30
14	26	132.3	1305	12.70	0.28	1.22	7.54	17.5	21.0	20
15	27	133.0	1475	11.89	0.27	1.20	7.48	20.5	20.5	15
Average.				**13.06**	**0.27**	**1.24**	**7.94**			
Average.	18–27			**12.45**	**0.29**	**1.22**	**7.33**			
16	28	133.3	1350	11.75	0.31	1.22	6.64			
17	29	133.3	1278	11.25	0.31	1.10	6.70			
Average.				**11.50**	**0.31**	**1.16**	**6.67**			

brief. The calorific value of the food as well as the constancy in body weight would suggest that the slightly higher excretion of nitrogen during the bathing periods is a result of stimulated metabolism, rather than a utilization of proteins to meet an increased need for fuel.

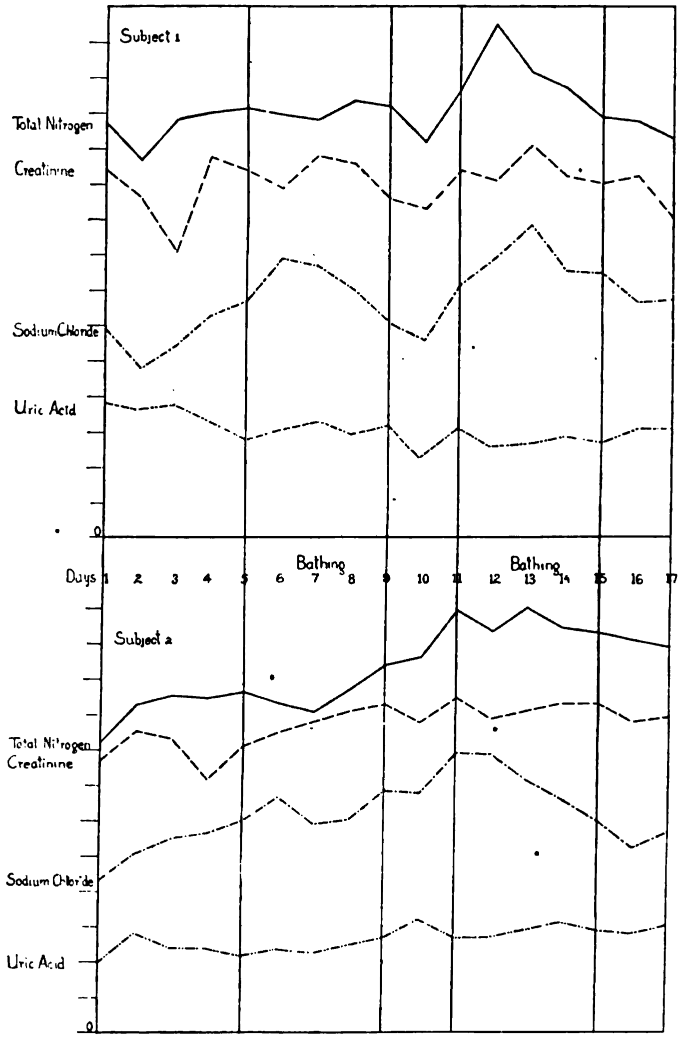

Fig. 1. Total nitrogen and chlorides are plotted in grams, uric acid and creatinine in tenths of grams, all to the same base line for each subject except creatinine in subject 1 which is dropped two spaces to avoid confusion.

Uric acid. The diet was practically purine-free, so that the uric acid may be considered as endogenous in origin. The variations in its elimination are too slight to indicate any effect of the bathing; to be complete these results should be supplemented by values for uric acid in blood which was not determined.

Subject 2

DAY OF EXPERIMENT	DATE	BODY WT.	URINE VOLUME	TOTAL N	URIC ACID	CREATININE	CHLORIDES	TEMP. OF AIR	TEMP. OF WATER	LENGTH OF BATH MINUTES
		Pounds	*c.c.*	*Grams*	*Grams*	*Grams*	*Grams*	*Cent.*	*Cent.*	
1	6/13	103.7	1238	8.21	0.20	0.77	4.31			
2	14	104.1	1177	9.27	0.28	0.85	5.07			
3	15	104.5	1288	9.54	0.24	0.83	5.51			
4	16	104.9	1148	9.45	0.24	0.72	5.66			
5	17	104.9	1318	9.63	0.22	0.81	6.01			
Average.				**9.22**	**0.24**	**0.80**	**5.31**			
6	18	106.0	1383	9.33	0.24	0.85	6.68	26.0	22.2	15
7	19	106.0	1207	9.08	0.23	0.88	5.94	31.0	23.6	25
8	20	106.3	1314	9.73	0.25	0.91	6.05	23.5	23.0	15
9	21	106.7	1471	10.43	0.27	0.93	6.87	27.0	24.5	30
Average.				**9.64**	**0.25**	**0.89**	**6.39**			
10	22	106.5	1130	10.66	0.32	0.88	6.82			
11	23	106.8	2015	11.97	0.27	0.95	7.95			
Average.				**11.32**	**0.30**	**0.91**	**7.39**			
12	24	106.5	1647	11.37	0.27	0.89	7.91	21.8	22.8	40
13	25	106.5	1600	12.03	0.29	0.91	7.17	20.5	22.5	25
14	26	106.4	1446	11.47	0.31	0.93	6.61	17.5	21.0	15
15	27	106.5	1343	11.31	0.29	0.93	6.01	20.5	20.5	10
Average.				**11.55**	**0.29**	**0.92**	**6.93**			
Average.	18–27			**10.74**	**0.27**	**0.91**	**6.80**			
16	28	106.7	1190	11.09	0.28	0.88	5.24			
17	29	107.1	1330	10.89	0.30	0.89	5.65			
Average				**10.99**	**0.29**	**0.89**	**5.45**			

Chlorides. An increased elimination of chlorides during the bathing period is evident, amounting to 20.95 per cent for Subject 1, and 27.10 per cent for Subject 2, over the average amounts eliminated during the preliminary and final periods. In Subject 1 these variations again follow the separate bathing

periods closely, while in Subject 2 the two-day interim without bathing shows a higher excretion than the bathing periods, both of which, however, show markedly higher values than preliminary and final periods. Regarding the absorptive powers of the human skin a considerable body of literature over long years fails to give conclusive evidence. It is generally held that watery solutions, not acting chemically on the epidermis, are not capable of absorption by the intact skin of man, while such fluids as can wet the skin, namely, fats and their solvents, may be imbibed by the cells, or may make their entrance through the capillary spaces between them (20). That the sebum is in fact the barrier to the entrance of aqueous solutions is made probable by the fact that after cleansing the skin with ether it is no longer impermeable to such solutions. Of particular interest are the results of Kahlenberg (21) who was able to show the rapid absorption of boric acid through the ether-cleansed skin of the feet. It is probable that the capillary spaces as well as the cells themselves may serve as channels for absorption, since rubbing on various ointments is more effective as far as absorption is concerned if the skin is rubbed in one direction only. Mucous surfaces did not furnish an avenue of entrance as special care was taken to avoid getting water into the mouth. As the skin was not rinsed off with fresh water following these brine baths considerable salt remained as a thin film and it is possible that, aided by the friction of the clothing, the very small salt particles might gradually make their way between the capillary spaces and so come to absorption.

To say that all of the augmented elimination of chloride came from salt absorbed through the skin would be to leave out of account the considerable stores of chloride over which the body has disposition. Such stores are made evident at the beginning of a fast when the chloride excretion for the first day or two is much above the level to which it later falls (22). The variations in this experiment might be considered as expressive of an increased catabolism of chloride-containing compounds. This increase is not shared by any other urinary constituent determined except total nitrogen; the parallelism in the total nitrogen and chloride curves is especially pronounced in Subject 1,

but the increase in protein catabolism is entirely inadequate to account for the increased chloride excretion (23). Further, it is usual in metabolism experiments to find chloride and nitrogen entirely independent of each other. If the parallelism is not accidental we have no sufficient explanation for it at present.

Creatinine. The creatinine variations, while very slight indeed, are at least consistent in showing increased excretion during the bathing periods, though here again Subject 2 shows a lag. Creatinine, as an end product of endogenous metabolism is generally unaffected, as to its amount, by muscular activity; muscular tonus, on the other hand, has been thought to be more closely related (24), and if this is so, and if the values obtained were significant, the question would be answered. Subsequent work during bathing in colder water[2] failed to support this idea.

[2] Creatinine determinations were made during a stay at the sea-shore; the water was considerably colder (15°C.) and the salt concentration, of course, much less. For reasons of convenience the subjects were not placed on a uniform diet, but since creatinine excretion is independent of all dietary constituents except the creatinine of meat, and since, on an ordinary uncontrolled meat diet the average variation from the non-meat diet is very slight, 0.05 gram (Shaffer, Am. J. Physiol., 22, 1908, 454), it was thought not necessary, inasmuch as any variations, in order to be attributed to bathing would have to be greater than those that might result from variations in a partially controlled diet. The results are given in the subjoined table, and show no more evidence of increased creatinine excretion following bathing than do the data of Table 1. The constancy of creatinine excretion even on a mixed diet is noteworthy.

	SUBJECT 1		SUBJECT 2	
Date	*Creatinine grams*	*Length of bath*	*Creatinine grams*	*Length of bath*
July 23	1.56		1.03	
July 24	1.50		1.14	
July 25	1.37		0.98	
July 26	1.48		0.96	
July 27	1.50		1.04	
July 28	1.48		0.98	
July 29	1.47	20 minutes	1.00	15 minutes
July 30	1.38	no bath	0.98	no bath
July 31	1.49	22 minutes	1.07	15 minutes
August 1	1.55	27 minutes	1.06	20 minutes
August 2	1.40	20 minutes	1.04	20 minutes
Average				
Non-bathing days	1.47		1.02	
Bathing days	1.48		1.04	

The higher values in this table as compared with Table 1 are not significant. The dichromate used as a standard, even though taken from a paraffined bottle, contained moisture which was not removed.

The examination of hourly samples might reveal variations which 24-hour samples do not show. Perhaps the great lowering of body temperature frequently produced experimentally in man and animals by exposure to cold might modify creatinine metabolism. So far as we are aware, no work has been published, showing the effects of lowered body temperature on creatinine excretion, although its variations in fevers and other pathological conditions are known.

TABLE 2

DATE	TIME	TEMPERATURE (FAHRENHEIT) Subject 1	Subject 2	REMARKS
June 19.....	4.20 p.m.	99.6	99.6	before bath
	4.50 p.m.	99.1	98.4	just after bath
	7.00 p.m.	99.4	99.5	after return to laboratory
June 21.....	1.35 p.m.	99.35	99.6	before lunch
	3.25 p.m.	99.4	99.75	before bath
	3.55 p.m.	99.15	98.3	immediately after bath
			98.0	3 minutes later
		98.4	97.95	10 minutes later
		99.2	99.7	after return to laboratory
June 24.....	3.45 p.m.	99.62	99.95	before bath
	4.35 p.m.	99.2	97.3	5 minutes after bath
			98.0	8 minutes after bath
		98.8	98.0	10 minutes after bath
		98.5		15 minutes after bath
June 25.....	3.40 p.m.	99.55	99.85	before bath
	4.15 p.m.	99.6	98.5	5 minutes after bath
		99.2	98.5	8 minutes after bath
		98.9		11 minutes after bath
		98.85		14 minutes after bath

Body temperature. The drop in temperature resulting from the bath was usually about 1° from Subject 1, and nearly 2° for Subject 2. Readings were taken only occasionally and while the temperature changes are not great some interest attaches to the measurements which are of the rectal temperature. Some of the results are given in Table 2.

Although the subjects stayed in the water until uncomfortably cold, the body temperatures immediately after bathing are

generally not the lowest reached, but the temperature continues to fall (Subject 1) for five to ten minutes, while the body is being dried with a towel and the usual clothing is being put on. This, we find, has been noted before (25). Probably the evaporation of such moisture as is not removed by drying with a towel is sufficient to account for this fall in body temperature.

SUMMARY

Two subjects were maintained on a uniform diet containing in one case 14.0, in the other 12.6 grams of nitrogen daily for seventeen days. A foreperiod of five days was followed by two bathing periods of four days each separated by an interim of two days without bathing. The lake water had a content of 19.2 per cent total solids of which 93 per cent were chlorides (as NaCl). The urine, collected in 24-hour periods, was analysed for total nitrogen, creatinine, uric acid and chlorides. Total nitrogen excretion in the two subjects shows an increase of 7 and 5 per cent during the bathing periods, over the amounts excreted in the preliminary and final periods, which is probably a true stimulation of nitrogen metabolism rather than a destruction of protein for fuel. Uric acid variations are small. Creatinine elimination shows a slight rise during the bathing periods, which, if significant, may be related to increased muscular tonus. Body temperature fell 1 to 2°F. as a result of bathing, falling most rapidly not during the bath but after it while drying. Chloride excretion was considerably increased during the bathing periods, in the two cases 21 per cent, and 27 per cent, over the amounts eliminated during the preliminary and final periods. These variations have no adequate parallel in those of any other catabolite determined and the possibility of absorption through the skin is suggested and discussed.

LITERATURE

(1) Umber: Lehrb. d. Ernährung und Stoffwechselkrankh., p. 329, quoted by v. Fürth, Probleme d. physiol. und pathol. Chem., **2**, p. 182.
(2) Bahrmann and Kochmann: Therap. d. Gegenw., n. F., **5**, 1903, 393–400.
(3) Garrod: quoted by v. Fürth, loc. cit.

(4) Hawk and Collaborators, J. Am. Chem. Soc., **32** and **33**, 1910–11.
(5) v. Noorden: Metabolism and Practical Medicine, **3**, pp. 958 and ff. (London, 1907).
(6) Formamek: Z. physiol. Chem., **19**, 1894, 271–281.
(7) Lepine and Flavard: Gazette med. de Paris, **6**, ii, 1880, 162; quoted by Formanek, loc. cit.
(8) Rubner: Arch. Hyg., **46**, 1903, 390; quoted by Lusk, Science of Nutrition, 2d ed., p. 10.
(9) Topp: Therap. Monatsh., **8**, 1894, 1; 55.
(10) Formanek: Monatsh. f. Chem., **13**, 1892, 476–481.
(11) Hawk and Mattill: unpublished.
(12) Heubner: Berl. klin. Wochsch., **42**, 1904, 489–493; 543–545.
(13) Lehmann: Adhesion of Bath Substances to the Skin. Diss. Bonn. 1876.
(14) Hiller: Z. klin. Med., **17**, 1890, Suppl., 257–306.
(15) Frankenhäuser: Berl. klin. Wochsch., **40**, 1903, 649.
(16) Schwenkenbecher: Das Absorptionsvermögen der Haut. Habilitationsschrift, Strassburg, 1904.
(17) Winternitz: Deut. Arch. klin. Med., **72**, 1902, 258.
(18) Loewy, Müller, Cronheim, Bornstein, Z. exp. Path. Ther., **7**, 1910, 627–685.
(19) Ebaugh and MacFarlane: Science, **32**, 1910, 568.
(20) Schäfer: Textbook of Physiology, **1**, p. 686.
(21) Kahlenberg, Private communication.
(22) Benedict: Infl. of Inanition on Metab. Carnegie Pub., **77**, 1907, 415.
(23) Rulon and Hawk: Arch. Int. Med., **7**, 1911, 542.
(24) Pekelharing and Harkink: Z. physiol. Chem., **75**, 1911, 207–215.
(25) Jürgensen: Deut. Arch. klin Med., **4**, 1868, 323–375.

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